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# **Hevea Germplasm in Vietnam: Conservation, Characterization, Evaluation and Utilization**

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## **1. Introduction**

Germplasm collections have provided original materials for plant breeding program and crop improvement. Because of their genetic diversity and possible occurrence of particular desirable genes, germplasm collections are useful targets for plant breeders as well as other biologists. Recently, many of germplasm are being lost worldwide due to habitat destruction, invasion of foreign species, and reliance on fewer high yielding strains. Therefore, maintaining germplasm of agricultural crops is very important. Normally, a germplasm collection will be utilized based on its characters of immediate perceived value or its potential variation. Furthermore, it can also be used to better understand the properties and performances of the plants, particularly at the genomic level. Nowadays, countries all over the world have set up facilities for conservation, characterization and utilization of germplasm collections of various crops either directly or indirectly.

Rubber tree (*Hevea brasiliensis* Willd. ex A. de Juss. Müell. Arg) which produces natural rubber is a tall deciduous perennial tree belonging to the Euphorbiaceae family. *Hevea brasiliensis* is a native of the Amazon basin and was introduced to countries in the tropical belts of Asia and Africa during late 19th century. It can be termed as one of the most far reaching and successful introductions in plant history, resulting in plantations with about 10.6 million hectares in the world for providing the industry with natural rubber of 9.62 million tonnes in the year 2009 (IRSG, 2009). Approximately 78% of the cultivation has taken place in Southeast Asia, 15% in other Asia countries, 5% in Africa, and 2% in Latin America (IRSG, 2009). The main producing countries were Thailand, Indonesia, Malaysia, India and Vietnam (IRSG, 2010). As the fifth natural rubber producer in the world, Vietnam produced 723,700 tonnes that shared about 7.5% of the world's natural rubber production and total area under rubber trees were 674,200 hectares that shared 6.1% of the world's rubber area (Hoa, 2010). In Vietnam, areas under rubber trees are mainly in the South-eastern region (65.2%), followed by the Highlands (23.4%), central coastal area (9.7%) and the new areas developed in the North-western region (1.6%) (Hoa, 2010).

*Hevea brasiliensis* was introduced into Vietnam in 1897 from Bogor (Indonesia) by Alexandre Yersin. Since then, rubber tree has been considered as one of the most important crops and

widely cultivated throughout the country, particularly in the Southeast and Highlands regions of Vietnam. Currently, about 3,500 accessions of *Hevea brasiliensis* have been collected and conserved in Vietnam. The majority of this germplasm were derived from the IRRDB'81 germplasm collected in the Amazonian habitats of the genus. This collection has been considered as the key factor contributing to the improvement of rubber tree through breeding programs. The characterization and evaluation of the germplasm are considered to be important aspects of *Hevea* germplasm conservation. Without proper characterization and evaluation, valuable genetic variation in the collections cannot be used for rubber improvement effectively. Understanding the genetic diversity of different genetic resources of *H. brasiliensis* would be important in order to optimize their management and to utilize *Hevea* germplasm in breeding programs.

## 2. *Hevea* genetic resources

The genus *Hevea* is basically composed of 10 species: *H. brasiliensis*, *H. guianensis*, *H. benthamiana*, *H. pauciflora*, *H. spruceana*, *H. microphylla*, *H. rigidifolia*, *H. nitida*, *H. camporum*, and *H. camargoana* (Schultes, 1990). According to Clement-Demange et al. (2000), it is generally considered that there is no biological barrier between them, and some species proved to be inter-crossable by hand-pollination; therefore, the *Hevea* species can be considered as a species complex. *H. paludosa* has been identified in Brazil by Ule in 1905 as the eleventh species (Gonçalves et al., 1990; Priyadarshan and Gonçalves, 2002). A review on the elaborate description of taxonomical and botanical aspects of *Hevea* has been reported by Schultes (1977, 1987, 1990) and Wycherley (1992). As its natural habitat, *Hevea* species have presented in Brazil, Bolivia, Peru, Ecuador, Colombia, Venezuela, Surinam and French Guiana as shown in Figure 1. These *Hevea* species have  $2n = 36$  chromosomes, with the possible exception of one triploid clone of *H. guianensis* ( $2n = 54$ ) and the possible existence of one genotype of *H. pauciflora* with  $2n = 18$  (Baldwin, 1947; Majumder, 1964), and *H. brasiliensis* behaves as an amphidiploid (Ramaer, 1935; Ong, 1975; Wycherley, 1976).

It is known that all high-yielding cultivars of rubber tree (*Hevea brasiliensis* Muell. Arg.) in the world originated from breeding programs initially developed in Southeast Asia with a very narrow genetic base. Historically, the introduction of the rubber trees into Asia began with the transfer of 70,000 seeds to England by Henry Wickham in 1876 (Wycherly, 1968). During the years of 1876-1877, a total of 2,397 *Hevea* seedlings were sent to several Asian countries such as Sri Lanka (1,919 seedlings), Bogor (Java) (18 seedlings) and Singapore (22 seedlings) (Wycherly, 1968; Dean, 1987; Baulkwill, 1989). Because of the centrally geographical position of Singapore in Asia and the influences of the British to the development of the rubber industry during these years, the collection of 22 seedlings planted in the Singaporean botanical garden became the main source of the rubber trees which was distributed to and planted in Asian countries later. Since this introduction, the rubber tree has become an important perennial crop as the major source of commercial rubber in the world (Fig. 2) and this collection was mentioned as Wickham collection (W). Since the current *Hevea* varieties all came from such a single population, it is necessary to enlarge the genetic basis for *Hevea* breeding program.

In order to enlarge the genetic basis for *Hevea*, a large collection of *H. brasiliensis* accessions from various areas in Colombia was gathered by Schultes after 1945 and then duplicated in

Ivory Coast (Nicolas, 1985). In addition, a collecting survey in the Madre de Dios basin in Peru was organized in 1948 by the Peruvian Ministry of Agriculture (Rands and Polhamus, 1955); the resulting seedlings were multiplied by grafting, and then introduced and studied in Liberia, Guatemala and Brazil under the name of MDF accessions (Bos and McIndoe, 1965). Moreover, another collecting expedition was carried out in the Brazilian states of Acre and Rondonia by both the Brazilian Agricultural Research Corporation (EMBRAPA) and French Institute for Rubber Research (IRCA) in 1974 (Hallé and Combe, 1974; Seguin et al., 2003); 42 resulting wild elite-tapped trees were collected and propagated by grafting under the name as EMBRAPA/IRCA accessions. Remarkably, in 1981, the International Rubber Research and Development Board (IRRDB) conducted an expedition covering three western states of Brazil, namely Acre (AC), Rondonia (RO), and Mato Grosso (MT), in 16 different districts and in 60 different locations overall to collect wild *Hevea* germplasm (referred as IRRDB'81 collection). As a result, a total of 63,768 seeds, 1,413 meters of budwood from 194 high yielding trees and 1,160 seedlings were collected (Tan, 1987; Simmonds, 1989; Onokpise, 2004). Of these, 12.5% and 37.5% of the seeds were sent to Malaysia and Ivory Coast, respectively, and the remaining 50% of the collections were maintained in Brazil (Clément-Demange et al., 2007). The genotypes issued from budwood collection were also then brought to Malaysia and Ivory Coast. The collection planted in Malaysia and Ivory Coast has then been distributed to all IRRDB members as clones since 1984.



- ..... Distribution area of *Hevea*
- Distribution area of *Hevea brasiliensis*

Fig. 1. Geographical origin of *Hevea* (adapted from George (2000))



Fig. 2. Distribution of rubber tree (*H. brasiliensis*) in the world where this species has been planted (adapted from Orwa et al. (2009)). Native range: Bolivia, Brazil, Colombia, Peru and Venezuela; Exotic range: Brunei, Cambodia, China, Ethiopia, India, Indonesia, Laos, Liberia, Malaysia, Myanmar, Philippines, Singapore, Sri Lanka, Thailand, Uganda, Vietnam, Guatemala, Cameroon, Ivory Coast, Ghana, Gabon, Guinea, Liberia, Nigeria, Congo, Bangladesh, Papua New Guinea, and Mexico.

### 3. Long-term conservation of *Hevea* germplasm

*In situ* and *ex situ* conservations are the two major strategies used in the conservation of plant genetic resources. *In situ* conservation, the conservation of diversity in its natural habitat, involves the designation, management and monitoring of the population at the location where it is currently found and within the community to which it belongs whereas *ex situ* conservation, collection of which the biodiversity is preserved outside its natural habitat, involves the sampling, transfer and storage of a population of a certain species away from the original location (Maxted et al., 1997). Because of a number of advantages, the *ex situ* method has been used to primarily conserve numerous plant genetic resources. In this method, plant diversity is safely preserved and concentrated in a small number of controlled places under consistent environmental conditions and is readily accessible to breeders.

An alternative solution to rubber tree *in situ* genetic conservation is the management of existing *ex situ* collections (Le Guen et al., 2009). Because of the ease of vegetative propagation by grafting, many *ex situ* collections of *Hevea* were established in various rubber producing countries. In Vietnam, the *ex situ* conservation of *Hevea* germplasm was established in 1985. This germplasm included the collection of local *Hevea* accessions, the introduction of *Hevea* clones from other countries. The majority of this germplasm collection was derived from the IRRDB'81 collection expedition in the Amazon forests of Brazil which is the primary center of diversity of the crop and the source of wild rubber trees. This germplasm was introduced into Vietnam in the form of budwood in 1984. All of the materials were sent to Lai Khe experimental station of the Rubber Research Institute of Vietnam (RRIV) in Binh Duong province, which is located in the traditional rubber growing belt. On receipt of the budwood, each accession was first multiplied by bud grafting and

then planted in the field genebank for conservation in the form of source-bush garden (Fig. 3). This source-bush garden was laid out in randomized complete block design, in which each accession was represented in two replications of five trees with a planting distance of 1.5 m x 1.2 m. The genetic resources of *Hevea* germplasm conserved in Vietnam were showed in Table 1. The source-bush garden is cut back every year to maintain the conservation and also to generate budwood for various evaluation trials. Several preliminary field evaluation trials for most of *Hevea* germplasm accessions have been being established in several representative locations to evaluate their agronomical and morphological characteristics.



Fig. 3. The source-bush garden for *ex situ* conservation of *Hevea* germplasm in Vietnam

Genetic sources	Number of accessions
<b>South America</b>	<b>3082</b>
Acre (AC)	959
Mato Grosso (MT)	901
Rondonia (RO)	1116
Others	106
<b>Africa</b>	<b>38</b>
Ivory Coast	38
<b>Asia</b>	<b>422</b>
Vietnam	338
Malaysia	55
Indonesia	7
Sri Lanka	16
China	5
Cambodia	1
<b>Total</b>	<b>3542</b>

Table 1. The genetic resources of *Hevea* germplasm conserved in Vietnam

#### 4. Characterization and evaluation of *Hevea* germplasm

In order to use the germplasm in breeding programs, it must be characterised and evaluated. There is often a delay between collection of germplasm and its evaluation, particularly for rubber trees because of the time required for them to reach maturity. Evaluation is useful if it considers the traits wanted by plant breeders. We are fortunate that our program of germplasm acquisition and evaluation is very closely linked to our program of *Hevea* breeding with the same people usually involving in both. Preliminary evaluation can help indicate those accessions that need more detailed evaluation, but those that appear not to be of immediate values should not be simply discarded.

##### 4.1 Agronomical characteristics

Standard characterization and evaluation of germplasm collection may be routinely performed using different methods including traditional practices such as the use of descriptive lists of morphological characters. They may also involve evaluation of the agronomical performances under various environmental conditions. Understanding the nature and the magnitude of variability of important traits existing among plant genetic materials is vital for the effective utilization of such materials for breeding purposes. In rubber tree, high latex yield is always the exclusive objective of breeding programs. To this ultimate objective, many different factors are associated. For instance, the main components of productivity are the growth of the trunk determined during the immature period before the beginning of tapping, the resistance to various diseases and the tolerance to stress factors such as high altitude, low temperature, wind damage and moisture deficit.

In Vietnam, a part of *Hevea* germplasm, especially IRRDB'81 collection, has been agronomically evaluated in various clonal trials including arboreta and small scale clonal trials at different locations. In the view of latex production, IRRDB'81 collection exhibited very poor performance with an average latex yield of around 16% of the level of the currently developed Wickham clones after 5 years of tapping. This result was similar to that of other studies conducted on IRRDB'81 collections in Malaysia, Indonesia, Ivory Coast and China (Ramli et al., 2004; Aidi et al., 2002; Clément-Demange et al., 2002; Hu et al., 2002). Outstandingly, in the first three years of tapping, some IRRDB'81 accessions such as AC56/276, AC62/54, MT8/27, MT/I/2 and RO62/26 produced 30.0 - 45.0 gram/tree/tapping, or 102.7 - 153.8% of the production of the control clone (GT1). These accessions have been being used as parents in *Hevea* breeding programs. Considering geographical origins, accessions derived from Mato Grosso seemed to be better yielder than those from Acre and Rondonia. This might indicate their better adaptability to the experimental areas where the climate featured by a distinct dry spell of six months is similar to that of the original region which is known to have a dry spell of three to four months annually (Chevallier, 1988). A large number of IRRDB'81 accessions showed very good growth performance with girth at opening ranging from 59.9 - 74.0 cm, or 124.8 - 133.5% of that of the control clone. Remarkably, some IRRDB'81 accessions such as MT29/68, RO24/58 and RO32/104 had significantly higher girth than the control when planted in the highland area which was considered as a non-traditional rubber region in Vietnam. The superiority of these accessions will be of considerable value in advanced breeding programs.

Recently, IRRDB'81 collection has been considered as an important source for timber selection and rubber wood production. The average wood volume of the IRRDB'81

collection conserved in Vietnam was initially recorded, yielding 1.48 m<sup>3</sup>/tree at the age of 21 years, 43.4% higher than the Wickham population. Among these, several accessions appeared to be the best with clear bole volume ranging from 1.55 to 2.07 m<sup>3</sup>/tree, which could be considered as suitable clones for timber production purposes. Similarly, several high timber yield accessions of the IRRDB'81 collection in Indonesia and Malaysia were also reported. At the age of 13 years, a total of 28 accessions in Indonesia and 20 accessions in Malaysia were selected for timber yield with clear bole volume at a range of 0.90 to 2.56 m<sup>3</sup>/tree (Aidi et al., 2002) and 1.0 to 1.6 m<sup>3</sup>/tree (Ramli et al., 2004), respectively.

It is known that unlike other clonally multiplied species, *Hevea* is not affected by viral diseases (Simmonds, 1989). Other diseases which are considered as economic importance are *Gloeosporium* leaf disease (*Colletotrichum gloeosporioides* Pen. Sacc.), pink disease (*Corticium salmonicolor* Berk. & Br.), powdery mildew (*Oidium heveae* Stein.), *Corynespora* leaf fall (*Corynespora cassiicola* Berk. & Curt. Wei.), *Phytophthora* leaf fall (*Phytophthora* sp.) and SALB (South American Leaf Blight - *Microcyclus ulei* P. Henn von Arx.). Among these diseases, *Corynespora* leaf fall and SALB are the most important in rubber plantations. Wycherly (1969) noted that the clonal and location specificity was evident towards resistance to these diseases. Differences in the level of resistance to important diseases have been observed in IRRDB'81 collections from different countries. In India, a large number of IRRDB'81 accessions were showed to be resistant to powdery mildew (140 accessions) and *Corynespora* leaf fall (70 accessions) (Varghese et al., 2002). Similarly, a total of 21 accessions in IRRDB'81 collection are resistant to powdery mildew in bush-wood garden in China (Huang et al., 2002). The resistance to SALB was observed on 298 accessions from *ex situ* germplasm collection planted in both French Guyana and Brazil, of which the accessions from Acre and Rondonia were the most resistant (Le Guen et al., 2002). In Vietnam, it seemed that Wickham and IRRDB'81 accessions showed no significant difference in susceptibility to pink disease, powdery mildew and *Gloeosporium* leaf diseases (Lam et al., 2002). In general, evaluations of IRRDB'81 collection for biotic and abiotic stresses are in progress in major rubber growing countries.

#### 4.2 Genetic diversity based on biochemical and molecular markers

In conventional plant breeding, many morphological traits have been used as markers for genetic analyses and cultivar identification, but specific genetic information on Mendelian traits are rare in *Hevea*. In contrast to traditional practices, genetic characterization refers to the description of the attributes that follow Mendelian inheritance or involve specific DNA sequences. In this way, biochemical assays, which detect differences between isozymes, or molecular markers were applied to determine the genetic diversity of the germplasm. In addition, the development of molecular and biochemical markers help researchers not only to identify genotypes, but also to assess and exploit the genetic variability (Whitkus et al., 1994). Insights into the relative genetic diversity among *Hevea* collections would be useful in *Hevea* breeding as well as *ex situ* conservation of *Hevea* genetic resources. The commercial value associated with identifying useful traits, especially yield and growth, would create a direct value in genebanks, ensuring long-term preservation of a collection. Moreover, *Hevea* germplasm characterization using molecular and biochemical markers will contribute to the knowledge of genetic relationships not only among wild accessions but also between accessions of wild and cultivated gene pool, and hence help to facilitate the breeding programs. In Vietnam, significant progress has been made in evaluation and



characterization of *Hevea* germplasm by applying genetic markers. Among several efficient methods to reveal the genetic variability within and among plant populations, the most widely applied methods are isozyme electrophoresis and random amplified DNA polymorphism (RAPD). Both markers are useful to analyze genetic diversity of *Hevea* germplasm, and to select good *Hevea* clones for future breeding or cultivation purposes.

#### 4.2.1 Isozymes marker

As first described by Hunter and Markert (1957), isozymes were defined as the different molecular forms in which proteins may exist with the same enzymatic specificity (Buth, 1984). This means that different variants on the same enzymes have identical or similar functions and are present in the same individual. Isozyme had played a minor role in research on plant biochemistry until genetic polymorphism for isozymes within the same population was discovered in 1966 (Stebbins, 1989; Wendel, 1989). In the 1980s, analysis of isozymes was developed at CIRAD (French Agricultural Research Centre for International Development) with 13 polymorphic isozymic systems to formulate a diagnostic kit associated with a clonal identification database. This kit has proved to be able to differentiate a large set of cultivated clones (Leconte et al., 1994). Since then, isozymes have been used as genetic markers for identification of rubber tree cultivars, genetic diversity analysis, controlling progenies issued from hand pollination and reproductive biology (Chevallier, 1988; Leconte et al., 1994; Paiva et al., 1994; Sunderasan et al., 1994). The first study on using isozyme markers for a precise understanding of the genetic diversity of the wild *Hevea* germplasm was carried out using ten isozyme markers on a set of 263 accessions from the IRRDB'81 collection (Chevallier, 1988).

Isozyme markers were firstly used in Vietnam to identify and confirm rubber clones in budwood gardens. Moreover, it was also used in *Hevea* breeding for hybrid genealogical legitimacy and genetic diversity research of *Hevea* germplasm. A total of 12 isozyme systems were used in studying genetic diversity of the IRRDB'81 *Hevea* germplasm in Vietnam. Banding patterns of representative IRRDB'81 accessions generated by isozyme electrophoresis are shown in Figure 4.

The study was performed on both IRRDB'81 collection and Wickham population with 117 accessions from 15 districts of the states of Acre, Rondonia and Mato Grosso of Brazil and 24 Wickham's clones (Fig. 5 and Table 2). The quantity of accessions sampled for each district was more or less proportional to the quantity of accessions currently conserved for the district. The result of isozymatic analysis showed that a total of 60 alleles were detected (Table 2). Out of them, 60 alleles were detected in IRRDB'81 accessions and 26 alleles in Wickham population. The result showed that the alleles detected in Wickham population were also detected in the IRRDB'81 collection, many new alleles were found in the IRRDB'81 collection only, thus underlining the genetic enrichment provided by the wild *Hevea* collections. Among IRRDB'81 collection, Acre accessions had the largest number of polymorphic alleles (51/60 alleles), followed by the Rondonia (45/60 alleles) and Mato Grosso accessions (45/60 alleles). This result revealed that Mato Grosso was obviously less polymorphic than Rondonia because Mato Grosso and Rondonia groups had the same number of detected polymorphic alleles but the volume of samples of the Mato Grosso groups was bigger than that of the Rondonia groups. The result also showed the significant polymorphic differences among the districts. The number of alleles detected in the districts Brasileira, Taurauca of Acre and district Cartriquacu of Mato Grosso was high in spite of the

small volume of the samples whereas the number of alleles detected in districts Itanba and Aracatuba of Mato Grosso was quite low.

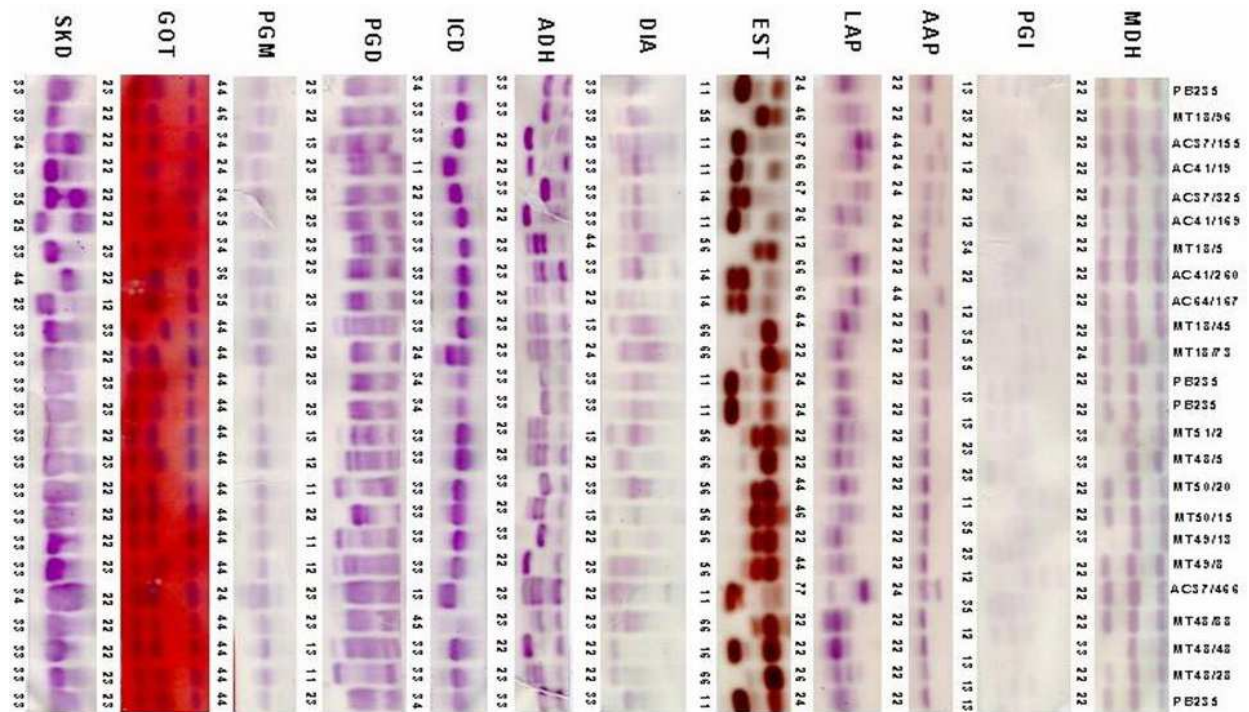


Fig. 4. Banding patterns of representative *Hevea* accessions generated by isozyme electrophoresis. Twelve isozyme systems used in studying genetic diversity of the IRRDB'81 *Hevea* germplasm had very different banding patterns. MDH, malate dehydrogenase; PGI, phospho glucose isomerase; AAP, alanyl amino peptidase; LAP, leucine amino peptidase; EST, esterase; DIA, diaphorase; ADH, alcohol dehydrogenase; ICD, isocitrate dehydrogenase; PGD, phosphogluconase dehydrogenase; PGM, phosphoglucomutase; GOT, glutamate oxaloacetate transaminase; SKD, shikimate acid

The study affirmed that the genetic base of *Hevea* germplasm in Vietnam, especially IRRDB'81 collection, was prosperous and diversified. Because of the high genetic variability level, this collection would contribute effectively to the long term progress of *Hevea* breeding and selection program in the country. In contrast, the Wickham population showed a low level of genetic variability, which is the consequence of the oriented selection through many years in a narrow geographical origin.

The result of cluster analysis based on isozymes database revealed the noticeable relationship between genetic clusters (Fig. 6). According to the genetic distance between the accessions, Acre accessions and Rondonia accessions were close to each other, meanwhile the genetic distance between Acre or Rondonia accessions were far from Mato Grosso accessions, except that the accessions from Vila Bella district of Mato Grosso (MT/VB) were close to those of Rondonia. Among IRRDB'81 accessions, Mato Grosso population was relatively close to Wickham collection based on isozyme analysis. In general, the genetic distance between the accessions conformed to the geographical origins of *Hevea*. However, several accessions of Arce and Rondonia were not separated distinctly using isozyme electrophoresis although they were distributed widely according to the geographical origins.

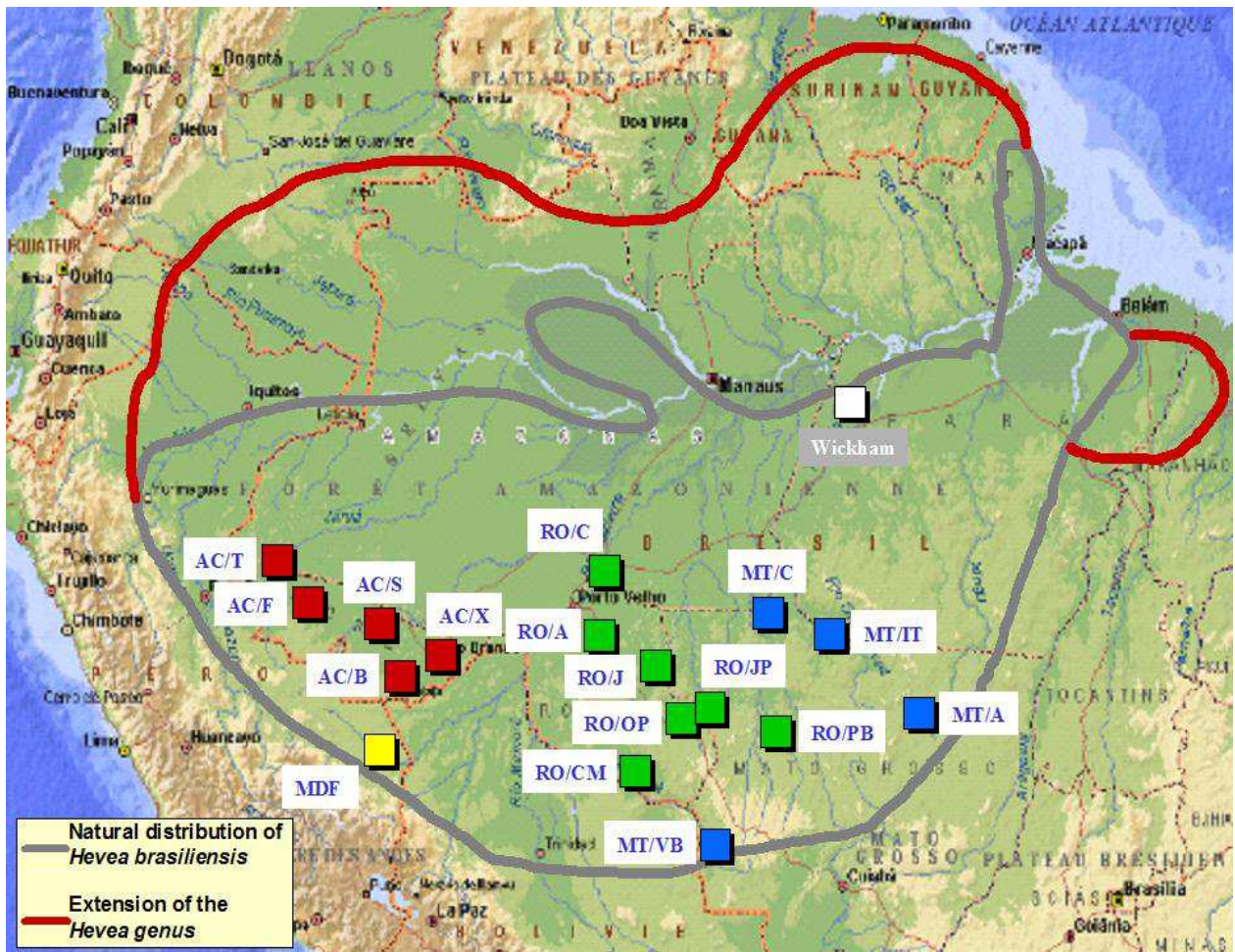


Fig. 5. Geographical origins of *Hevea* IRRDB'81 collection

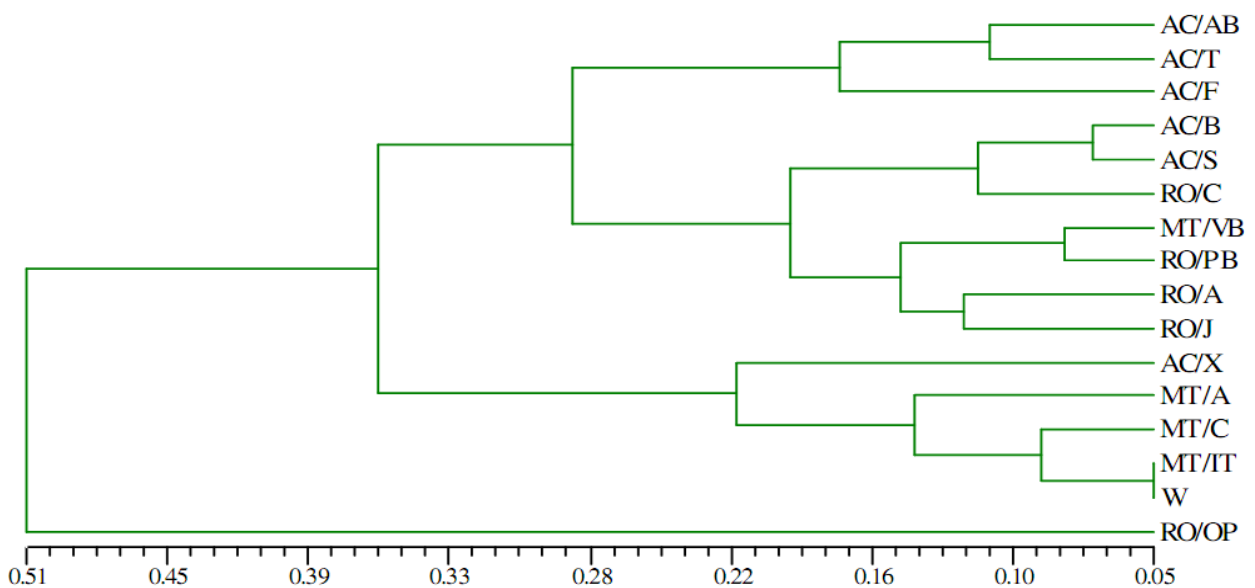


Fig. 6. Dendrogram based on Nei's genetic distance between IRRDB'81 collection and Wickham population.

Genetic resources	Number of accessions	Number of alleles/locus*												Total of alleles
		MDH	PGI	AAP	LAP	EST	DIA	ADH	ICD	PGD	PGM	GOT	SKD	
<b>IRRDB'81</b>	<b>117</b>	4	5	5	7	7	5	2	5	3	6	4	7	<b>60</b>
<b>Acre</b>	<b>58</b>	4	5	3	6	6	5	2	4	3	6	3	4	<b>51</b>
<i>Assis-brasil</i>	2	1	2	2	3	2	1	2	2	2	2	1	3	23
<i>Brasileia</i>	9	3	3	2	3	4	3	2	4	3	5	2	3	37
<i>Feijo</i>	15	3	3	2	4	3	4	2	4	3	4	2	4	38
<i>Sena Madureira</i>	21	2	5	2	4	4	3	2	4	3	6	3	4	42
<i>Tarauaca</i>	8	3	3	2	3	2	2	2	3	2	4	1	3	30
<i>Xapuri</i>	3	1	2	1	3	2	2	2	1	3	4	3	2	26
<b>Mato Grosso</b>	<b>32</b>	3	5	1	6	5	4	2	4	3	4	3	5	<b>45</b>
<i>Aracatuba</i>	9	2	4	1	3	3	3	2	3	3	1	2	1	28
<i>Cartriquacu</i>	7	2	5	1	5	5	4	2	3	3	3	3	1	37
<i>Itanba</i>	13	2	3	1	4	3	2	2	3	2	2	3	2	29
<i>Vila Bela</i>	3	1	3	1	1	3	1	1	2	2	3	1	4	23
<b>Rondonia</b>	<b>27</b>	3	5	4	4	4	2	2	4	2	5	3	7	<b>45</b>
<i>Ariquemes</i>	5	1	2	3	2	3	2	2	2	2	5	2	4	30
<i>Calama</i>	18	2	5	1	3	4	2	2	4	2	5	3	6	39
<i>Jaru</i>	2	1	1	1	1	2	1	2	2	2	2	1	3	19
<i>Ouro Preto</i>	1	1	1	2	1	2	1	1	2	1	2	1	2	17
<i>Pimenta Bueno</i>	1	2	2	1	1	2	1	1	1	2	2	1	2	18
<b>WICKHAM</b>	<b>24</b>	2	3	1	3	3	1	2	4	3	1	2	1	<b>26</b>
<b>TOTAL</b>	<b>141</b>	4	5	5	7	7	5	2	5	3	6	4	7	<b>60</b>

\* MDH, malate dehydrogenase; PGI, phospho glucose isomerase; AAP, alanyl amino peptidase; LAP, leucine amino peptidase; EST, esterase; DIA, diaphorase; ADH, alcohol dehydrogenase; ICD, isocitrate dehydrogenase; PGD, phosphogluconase dehydrogenase; PGM, phosphoglucomutase; GOT, glutamate oxaloacetate transaminase; SKD, shikimate acid

Table 2. Genetic variability of *Hevea* germplasm based on isozymes

The results of isozymes analysis indicated that the *Hevea* germplasm conserved in Vietnam is very diversified. This characterisation would help to utilize the new genetic resources more effectively in *Hevea* breeding programs. The combination of morphological characterization and isozyme markers could help breeders to constitute a core collection of *Hevea* IRRDB'81 germplasm to ensure the conservation of the genetic variability. In addition, maintaining the genetic variability in *Hevea* germplasm would help to reduce gene erosion. Moreover, isozyme markers could be used as an assistant tool to orient a long term plan to advance heterosis to improve Wickham materials based on the recombination between Wickham clones and IRRDB'81 accessions. However, isozyme-based analysis is limited by the rather small number of marker loci available and a general lack of polymorphism for these loci. In addition, the analysis has to be carried out near the field sites owing to the fragility of the isozymes to varied temperatures or otherwise the samples need to be freeze-dried and transported to the laboratory. In spite of such limitations, isozyme was still a helpful marker to evaluate the genetic variability of the *Hevea* germplasm in Vietnam.

#### 4.2.2 RAPD marker

The random amplified polymorphic DNA (RAPD) technique, first described by Williams et al. (1990), despite some limitations, has provided a useful approach for evaluating population's genetic differentiation, particularly in species that are poorly genetically known (Silva and Russo, 2000; Nybom, 2004). Recently, a large number of studies have pointed out that DNA-based markers, such as RAPD, were superior to isozymes in detecting genetic diversity (Garkava et al., 2000; Matos et al., 2001; Ochiai et al., 2001; Sharma et al., 2008). It is known that isozymes represent allelic expression of the same locus, while DNA fragments produced by RAPD are independent genetic markers (Ochiai et al., 2001) with a lower proportion of non-neutral markers than formerly (Bartish et al., 2000). Hence, isozyme and RAPD analyses often give discordant patterns, suggesting the importance of using multiple molecular marker systems in studies of population structure (Wendel and Doyle, 1998; Bartish et al., 2000; Lebot et al., 2003). RAPD marker was also used to evaluate the levels of gene flow between species (Arnold et al., 1991) and detection of gene introgression in various plant species (Waugh et al., 1992; Orozco-Castillo et al., 1994; Gomez et al., 1996). In rubber tree, RAPD has become a useful marker for investigating genetic diversity within and between *Hevea* populations, especially the IRRDB'81 collection (Varghese et al., 1997; Venkatachalam et al., 2002; Lam et al., 2009). Moreover, this marker was also used to identify a dwarf genome-specific marker (Venkatachalam et al., 2004) or certain homology to proline-specific permease gene (Venkatachalam et al., 2006) in rubber tree. The accumulated data on *Hevea* RAPD analysis from different accessions give information on genetic relations and *Hevea* origin, and provide the initial basis for clonal distinction and germplasm evaluation of agronomical interest. Therefore, the data can also be used in *Hevea* improvement programs.

In Vietnam, RAPD was firstly used to study genetic diversity of *Hevea* germplasm (Lam et al., 2009). The study was performed on IRRDB'81 collection with 59 accessions from 13 districts of the states of Acre, Rondonia and Mato Grosso of Brazil (Fig. 5). Using 6 oligonucleotide primers, the percentage of polymorphic loci calculated for individual districts ranged from 15.38% in Assis-Brasil district to 70.77% in Sena Madureira district of Acre, which totally had 10 and 46 polymorphic banding patterns, respectively (Table 3, Fig. 7). Although the sample sizes might have certain effects on the extent of the polymorphism of various districts, in the cases of Assis-Brasil of Acre, Ariquemes of Rondonia, and Vila Bela of Mato Grosso, they were quite different in the extent of polymorphism with the same sample sizes. In addition, the Jiparana district of Rondonia was very polymorphic regardless of its small sample size.

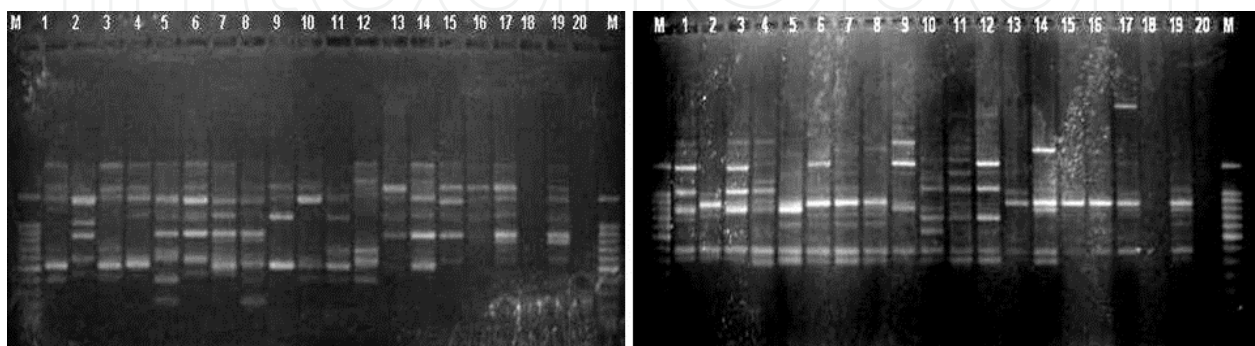


Fig. 7. DNA fingerprints of representative IRRDB'81 accessions generated by primer A18 (left) and OPB-12 (right)

State/District	No. of accessions	No. of total/polymorphic band patterns	Observed number of alleles	Mean heterozygosity	Shannon index	Genetic distance*
<b>Acre</b>	<b>25</b>					
Assis-brasil	2	41/10	1.154	0.064	0.093	0.139
Brasileia	4	52/37	1.569	0.223	0.328	0.299
Feijo	6	56/42	1.646	0.240	0.355	0.282
Sena Madureira	9	58/46	1.708	0.246	0.368	0.256
Tarauaca	4	56/32	1.492	0.196	0.287	0.225
<b>Mato Grosso</b>	<b>21</b>					
Aracatuba	6	51/38	1.585	0.227	0.333	0.264
Cartriquacu	5	51/38	1.585	0.222	0.328	0.306
Itanba	8	56/42	1.646	0.231	0.344	0.264
Vila Bela	2	46/21	1.323	0.134	0.195	0.296
<b>Rondonia</b>	<b>13</b>					
Ariquemes	2	45/16	1.246	0.102	0.149	0.216
Calama	4	53/36	1.554	0.217	0.319	0.290
Jaru	4	51/39	1.600	0.239	0.348	0.323
Jiparana	3	54/44	1.677	0.264	0.389	0.469
<b>Total/Mean</b>	<b>59</b>	<b>65/62</b>	<b>1.522</b>	<b>0.200</b>	<b>0.295</b>	<b>0.279</b>

\* Genetic distance is mean genetic distance among accessions in individual district

Table 3. Patterns of genetic diversity of *Hevea* IRRDB'81 collection based on RAPD analysis

In general, the IRRBD'81 collection conserved in Vietnam showed the high level of genetic diversity detected by RAPD. In fact, the mean values of heterozygosity or Nei's genetic diversity (Nei, 1978) within individual districts varied from 0.064 to 0.264 over 65 loci with the average of 0.2 across the districts (Table 3). This parameter differed substantially among 13 districts studied. The Jiparana district of Rondonia showed the highest estimated heterozygosity over 62 polymorphic loci, whereas the Assis-Brasil district of Acre showed the lowest one. In spite of high standard errors of mean heterozygosities, probably due to the small sample sizes, recorded in Assis-Brasil (Acre), Ariquemes (Rondonia), and Vila Bela (Mato Grosso) populations, the remarkable variations of mean heterozygosity clearly showed differences in genetic variability among 13 districts. The average degree of diversity within individual districts using Shannon's diversity index (Shannon and Weaver, 1949) was 0.296 and ranged from 0.093 for the Assis-Brasil district of Acre to 0.389 for the Jiparana district of Rondonia (Table 3). Shannon index was correlated strongly with the percentage of polymorphic loci in a district. In fact, the districts with high Shannon's diversity index also exhibited the high percentages of polymorphic loci (Table 3). Similar to mean heterozygosity, differences in values of Shannon's diversity index also showed genetic differentiation among the districts. The largest average genetic distance among accessions within the districts was detected in the Jiparana district of Rondonia and the smallest was found in the Assis-Brasil district of Acre, of which the average genetic distance values were 0.469 and 0.139, respectively (Table 3). According to previous studies, high genetic diversity is usual in IRRDB'81 accessions (Chevallier, 1988; Besse et al., 1994; Lekawipat et al., 2003). All the accessions had unique RAPD genotypes. Nei's genetic distance values between pairs of districts ranged from 0.046 for Catriquacu and Itanba of Mato Grosso to 0.304 for Tarauaca of Acre and Aracatuba of Mato Grosso (Table 4). The dendrogram constructed by UPGMA cluster analysis showed that *Hevea* IRRDB'81 collection of 13 different districts were in five clusters with Ariquemes of Rondonia quite different from the others (Fig. 8).

Among the districts of Mato Grosso, Vila Bela was in the same cluster with the districts from Rondonia (Jaru and Jiparana), meanwhile other districts (Itanba, Catriquacu and Aracatuba) were grouped into one cluster. This showed that Vila Bela was quite different from other districts of Mato Grosso, which was also noted by other researchers using RFLP markers (Besse et al., 1994). The distribution of those districts in genetic cluster analysis seemed to conform to geographical origins of *Hevea* IRRDB'81 collection, except Calama district of Rondonia.

District	AC/AB	AC/B	AC/F	AC/S	AC/T	MT/A	MT/C	MT/IT	MT/VB	RO/A	RO/C	RO/J	RO/JP
AC/AB		0.147	0.201	0.146	0.160	0.297	0.244	0.236	0.202	0.286	0.188	0.236	0.266
AC/B	0.147		0.106	0.107	0.155	0.190	0.135	0.161	0.216	0.275	0.135	0.160	0.164
AC/F	0.201	0.106		0.105	0.145	0.125	0.115	0.129	0.213	0.230	0.107	0.144	0.150
AC/S	0.146	0.107	0.105		0.190	0.153	0.151	0.146	0.158	0.239	0.103	0.164	0.169
AC/T	0.160	0.155	0.145	0.190		0.304	0.227	0.234	0.193	0.236	0.230	0.205	0.212
MT/A	0.297	0.190	0.125	0.153	0.304		0.094	0.086	0.296	0.295	0.171	0.191	0.183
MT/C	0.244	0.135	0.115	0.151	0.227	0.094		0.046	0.202	0.286	0.125	0.145	0.141
MT/IT	0.236	0.161	0.129	0.146	0.234	0.086	0.046		0.189	0.289	0.147	0.145	0.147
MT/VB	0.202	0.216	0.213	0.158	0.193	0.296	0.202	0.189		0.233	0.123	0.146	0.149
RO/A	0.286	0.275	0.230	0.239	0.236	0.295	0.286	0.289	0.233		0.222	0.189	0.205
RO/C	0.188	0.135	0.107	0.103	0.230	0.171	0.125	0.147	0.123	0.222		0.137	0.172
RO/J	0.236	0.160	0.144	0.164	0.205	0.191	0.145	0.145	0.146	0.189	0.137		0.097
RO/JP	0.266	0.164	0.150	0.169	0.212	0.183	0.141	0.147	0.149	0.205	0.172	0.097	

Table 4. Nei's genetic distance among 13 districts of *Hevea* IRRDB'81 collection

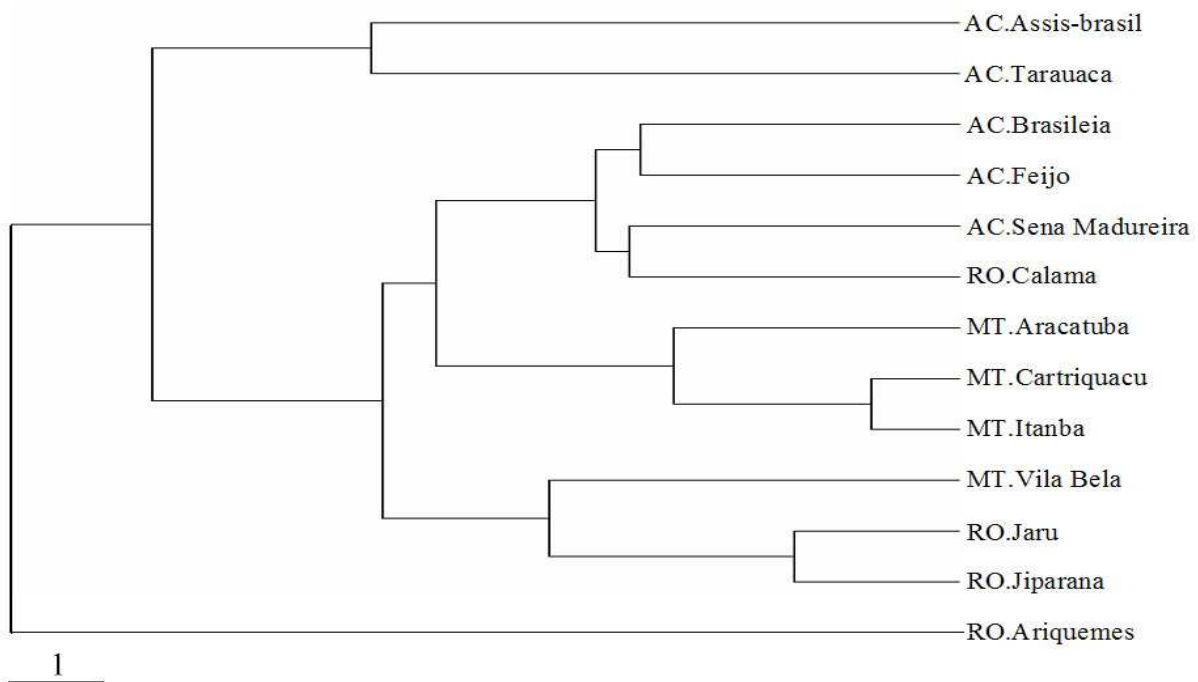


Fig. 8. Dendrogram of UPGMA cluster analysis based on Nei's genetic distance

Analysis of molecular variance (AMOVA) for 59 accessions of 13 districts revealed that the interpopulation (interdistrict) variation accounted for only 14.1% of the total genetic variance while intrapopulation (within district) variation accounted for 85.9% (Table 6). The results indicated that the majority of genetic diversity was within district variation (85.9%). The results suggests that there might be a certain gene flow among the districts, possibly owing to the species' outcrossing as a mode of reproduction and dispersion of seeds by a network of rivers in Amazon basin. However, it should be treated with caution due to small samples brought into the study.

Source of variation	df	SS	CV	%Total
Among districts	12	198.6	1.57	14.1
Within districts	46	439.6	9.56	85.9

Significant at  $P=0.001$

Table 5. Summary of the AMOVA analysis

The IRRDB'81 *Hevea* germplasm in Vietnam exhibited large genetic variability by RAPD markers. The intradistrict source accounted for most of the genetic variation of the germplasm. Based on the genetic distance analysis, the collection could be classified into five groups which could help in planning crosses based on genetic distance in the hope of looking for heterosis and maintaining the abundant genetic diversity. The RAPD markers could also help in checking the genetic variability of the *Hevea* breeding program. Moreover, in association with the agronomical characteristics, morphological traits and isozymes analysis, RAPD markers are now suitable tools for genetic diversity studies of *Hevea* germplasm and can be useful for accumulation and management of genetic-breeding resources of *Hevea brasiliensis*.

## 5. Utilization of *Hevea* germplasm in Vietnam

One of the major objectives of conservation of *Hevea* genetic resources is to make genetic diversity available for immediate or future use. The widest possible range of the genetic diversity has to be conserved in order to meet future, as yet unknown, needs. *Hevea* germplasm conservation program in Vietnam is expected to promote and facilitate the use of conserved materials through the maintenance of healthy and readily accessible and adequately characterised/evaluated materials, and proper documentation of the relevant information. Evaluation data of the agronomic performances and the morphological characteristics gathered during cultivation of the accessions have been being recorded continuously using a specifically constructed program (Fig. 9). Currently, a total of about 3,500 *Hevea* accessions have been collected and *ex situ* conserved. This germplasm comprises of three main genetic resources: the Amazonian (A) (most of which belong to IRRDB'81 collection collected in the Amazonian habitats of the genus), the Wickham (W), and the Wickham x Amazonian (WA) resources. The majority of this germplasm were derived from the IRRDB'81 collection with a total of 2,999 accessions, each of which is a clone derived from originally collected seedlings. Most of them have been evaluated for the agronomical and morphological characteristics. In the view of the limitations of the agronomical and morphological traits, isozyme and RAPD markers were used to analyze the genetic diversity and structure of the IRRDB'81 collection for more effective utilization of the germplasm in *Hevea* breeding programs in Vietnam.



Since the IRRDB'81 collection exhibited very poor profiles in agronomical characteristics, especially latex productivity, the chance for direct use of this collection for latex purposes seemed to be impractical although certain accessions could be planted for timber purposes. Regarding widening the genetic base for genetic improvement, several promising IRRDB'81 accessions have been included in hand pollination program in RRIV since 1997. Based on the agronomical and morphological traits as well as the genetic diversity analysis, recently, many attempts have been made to enlarge the genetic base of *Hevea* breeding materials by polycrossing among different genetic resources. In this way, many crosses between maternal



Fig. 9. Genebank documentation program in conservation of *Hevea* germplasm

W and paternal A accessions have been preferably made and the progenies of which are in various phases of evaluation with the expectation that the W x A progenies could combine the good agronomical characteristics from parents while optimizing the genetic variability in this population. In fact, Amazonian accessions were crossed with Wickham high yielding and good set fruit clones such as PB260 and RRIC110, and progenies derived from these hand pollination crosses were disbudded into fields of early selection trials for evaluation of agronomical performances such as latex yield, growth and diseases incidence. In general, these progenies exhibited rather good in girth but very poor in latex productivity. Most of the progenies produced a very little or negligible amount of latex; this agreed closely with the previous finding in Ivory Coast for the progenies from W x A crosses (Clement-Demange et al, 1990). However, some progenies had the production of 1.9 - 3.2 gram/tree/tapping or 106 - 132% of the production of the control clone (i.e. PB 260), and a large number of the progenies showed very good in growth performance with girth at 34 months old after planting ranging from 15.6 - 21.2 cm, or 101 - 161% of that of the control clone. These progenies are being further tested in the small scale clonal trials

and would be included as parents in future breeding programs. This gives a way for the opportunity of genetic improvement in breeding programs, particularly in latex productivity of the *Hevea* IRRDB'81 collection. In diseases incidence, all of the progenies exhibited varying degrees of infection to powdery mildew. The progenies derived from different paternal accessions showed significant differences in susceptibility to powdery mildew. For instance, the progenies derived from AC6/23 and AC35/114 were more susceptible to powdery mildew than other progenies. In contrast, the progenies derived from RO44/268 and RO44/71 seemed to be lightly susceptible to powdery mildew in comparison to other progenies. This result has contributed to the development of clonal disease resistance by genetic recombination using IRRDB'81 collection as paternal clones in *Hevea* breeding programs.

The results obtained so far can be considered as a basis to continue combining Wickham and IRRDB'81 genetic resources in breeding programs at RRIV. The W x A progeny population provides a valuable source for selecting multi-clones recommended for developing rubber cultivation in non-traditional regions in Vietnam.

## 6. Problems and challenges

### 6.1 Problems

Because the genetic structure of natural *H. brasiliensis* populations is based upon both hydrographical network patterns and long-range isolation by distance, it is likely that the known genetic diversity represents only part of its true natural diversity (Le Guen et al., 2009). In order to enlarge the genetic resources of *H. brasiliensis*, it is necessary to conduct additional expeditions in other areas that were not yet covered previously, such as Amazonian basin in Colombia, Peru and Bolivia, and the Brazilian states of Pará and Amazonas. Besides, since a very small part of the diversity of *Hevea* germplasm has been collected and conserved in Vietnam, a much greater diversity of the germplasm should be imported from other countries such as Brazil, Malaysia and Ivory Coast. Moreover, it is urgent need to duplicate the *Hevea* germplasm accessions, particularly the IRRDB'81 collection, in all IRRDB country members to prevent the loss of accessions and to increase the genetic resources as raw materials in *Hevea* breeding programs. Additionally, molecular tools may contribute in *ex situ* conservation of *Hevea* germplasm to the sampling, management and development of "core" collections as well as the utilization of genetic diversity. However, the use of such molecular tools is limited in the rubber growing countries including Vietnam due to their cost.

Rubber tree is traditionally propagated through bud grafting on unselected seedlings, which maintains intraclonal heterogeneity for vigour and productivity. Therefore, variation among a bud-grafted population is significant and can influence the growth and productivity levels. Therefore, a great improvement may be expected by using *in vitro* micropropagation. *In vitro* techniques have currently made a commercial impact in rubber, and their propagation systems can circumvent the influence of the stock-scion interactions in *Hevea* clones (Priyadarshan, 2007). Recently, there has been an increasing interest in the induction of somatic embryogenesis in rubber trees. However, successful somatic embryo formation and plant regeneration have been reported by a few researchers in different countries using limited genotypes of *Hevea*. In addition, the frequency of somatic embryo induction was

found to be very low and non-synchronous, its germination remains very difficult and thus *Hevea* embryogenic system needs further investigation. There has been no large scale commercial application of tissue culture techniques for mass propagation of clonal *Hevea* as yet, either by microcutting or by somatic embryogenesis. However, there is sufficient progress at the research level to suggest that tissue culture of *Hevea* can and should be further developed.

## 6.2 Challenges

At the scientific and technical levels, challenges are posed by genetic erosion, genetic vulnerability and utilization. Genetic erosion is defined as “the loss of genetic diversity, including the loss of individual genes, and the loss of particular combinations of genes (i.e. of gene-complexes) such as those manifested in locally adapted landraces” (FAO, 1997). There is no consensus on the optimal balance of *in situ* and *ex situ* conservation methods to combat genetic erosion (Fraleigh, 2006). A gradual erosion of the genetic variability of *Hevea* in all of natural rubber plantations was realized in the 1970s. This erosion occurred because most of *Hevea* clones in cultivation were derived from the few surviving seeds collected by Henry Wickham. Therefore, widening the genetic base of *Hevea* in production was seen as a prerequisite to generate new clones with new and valuable characteristics with regard to productivity, disease resistance, and tolerance to many environmental conditions.

Genetic vulnerability was described as “the condition that results when a widely planted crop is uniformly susceptible to a pest, pathogen or environmental hazard as a result of its genetic constitution, thereby creating a potential for widespread crop losses” (FAO, 1996). It is known that genetic vulnerability pertains to the level of the crop genetic diversity actually being used. Because of the very narrow genetic base in the commercially cultivated *Hevea* clones, the commercial rubber cultivation, due to their genetic vulnerability, is under a constant threat of attack by native as well as exotic diseases and insect pests. The changes in the weather parameters due to the increasing trend in climate change have further complicated the above issues. Climate change, which is clearly felt in the traditional rubber growing regions of Vietnam, may possibly alter the host-pathogen interactions. This will lead to the emergence of otherwise minor disease, and *Corynespora* leaf fall disease may represent this scenario. This pathogen is rapidly progressing into new areas, thus highlighting the need for stronger and advanced resistance breeding approaches.

It is known that the potential uses and values of *Hevea* genetic resources need to be understood by characterizing, evaluating and documenting them. Methods still need to be developed to improve and facilitate productive utilization. Although biotechnological methods are now increasingly available to facilitate productive utilization of *Hevea* germplasm, not all countries have the capacity to use such new technologies.

Another set of challenges is posed for taking action. For instance, how the necessary cooperation can be organized among countries and among disciplines, particularly in order to link the conservation and the use of *Hevea* genetic resources, and how the resources which need to address these issues can be mobilized. Besides, cryogenic preservation of endangered seedling trees is yet another important aspect to be looked into urgently.

## 7. Conclusions and prospects

Vietnam has received a large share of the *Hevea* germplasm collection of the 1981 IRRDB expedition. A total of 2,999 wild accessions belonging to three states of Brazil *viz.*, Acre, Rondonia and Mato Grosso are being conserved. Systematic efforts are underway for conservation, characterization, utilization and documentation of these valuable genetic resources. In general, the genetic base of *Hevea* in Vietnam which is prosperous and diversified has been contributing effectively to the long term progress of *Hevea* breeding program in the country. As management of the germplasm is a herculean task, IRRDB'81 accessions have been evaluated in a phased manner. Studies in different sets of this germplasm have been carried out since 1985 onward to assess the extent of variability present in the collection for various agronomical characteristics such as latex yield, girth, wood volume, diseases incidence, biotic and abiotic stresses, in order to exploit them in the improvement programs. A large number of IRRDB'81 accessions are now in various evaluation stages. The evaluated IRRDB'81 collection displayed unimproved characteristics of a wild population and was far inferior to the Wickham clones in agronomical performances, especially latex productivity. However, this germplasm had a much broader genetic variability which can help in broadening the narrow genetic base of cultivated rubber and also in developing location specific rubber clones for cultivation in the marginal and non-traditional rubber regions of the country.

The genetic parameters obtained from isozyme and RAPD analyses indicated that the *Hevea* germplasm conserved in Vietnam exhibited large genetic diversity. The biochemical and molecular markers have also shown to be the effective techniques for breeders to manipulate the *Hevea* germplasm. These markers could be used to select the parents with far genetic distance aiming at enlarging the genetic variability in their progenies and could help in checking the genetic variability of the *Hevea* breeding program. Furthermore, information on the structure of genetic diversity could help establish global *Hevea* collections for long-term conservation with minimum maintenance activity, and help define working collections for medium-term targeted utilization and breeding purposes.

The conventional solution to the conservation of *Hevea* genetic resources has been the establishment of *ex situ* genebanks. *Ex situ* conservation is the only effective method for the long-term conservation of *Hevea* germplasm. *Ex situ* conservation may also represent a last resort for many species and varieties including *Hevea* that would otherwise die out as their habitats are destroyed. Moreover, the management of existing *ex situ* *Hevea* collections is an alternative solution to rubber trees *in situ* genetic preservation (Le Guen et al., 2009). The main benefit of *ex situ* conservation is to provide breeders with ready access to a wide range of genetic materials with useful traits. *Hevea* germplasms have their own share of problems. Although only a small proportion of *Hevea* genetic resources are actually used by breeders, partly because of the inadequate characterization of the accessions, the costs of characterizing, evaluating and cataloguing genetic resource materials need to be carefully considered. Because of the severe limitations faced by large germplasm collection, establishing a core collection of this germplasm is necessary to facilitate a speedy and more efficient evaluation, and to get a collection which is conserved better and exploited more effectively.

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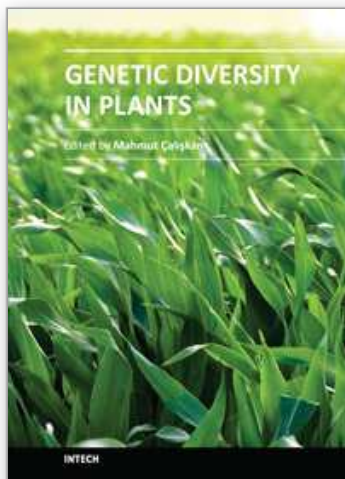
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Genetic diversity is of fundamental importance in the continuity of a species as it provides the necessary adaptation to the prevailing biotic and abiotic environmental conditions, and enables change in the genetic composition to cope with changes in the environment. Genetic Diversity in Plants presents chapters revealing the magnitude of genetic variation existing in plant populations. The increasing availability of PCR-based molecular markers allows the detailed analyses and evaluation of genetic diversity in plants and also, the detection of genes influencing economically important traits. The purpose of the book is to provide a glimpse into the dynamic process of genetic variation by presenting the thoughts of scientists who are engaged in the generation of new ideas and techniques employed for the assessment of genetic diversity, often from very different perspectives. The book should prove useful to students, researchers, and experts in the area of conservation biology, genetic diversity, and molecular biology.

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