Chapter 4
Dioscorea

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4.1 Introduction

After the cereal grains, majority of the world's population depends on root and tuber crops, which include stems, rhizomes, corms, bulbs, tubers, and other types of organs as well as true roots. Among these, yams – a multispecies crop – are considered as one of the most important staple food crops of sub-Saharan Africa that provide valuable source of dietary carbohydrate and income. They are mainly grown in tropical and subtropical Africa, Central and South America, parts of Asia, and the Caribbean and South Pacific Islands (Coursey 1967; Adelusi and Lawanson 1987). Yams are monocotyledonous classified under the genus *Dioscorea*, family Dioscoreaceae, and order Dioscoreales. More than 600 species have been reported in the genus *Dioscorea* (Coursey 1969), which has been reestimated to comprise about 250–400 species distributed throughout the tropics and subtropics growing wildly as climbing vines (Caddick et al. 2002). Less than 50 species have been domesticated for food and industrial use (Hahn 1995), of which only ten are important as staples in the tropics (Coursey 1969; Hahn 1995), while many of the wild species are also a reliable source of food during food scarcity. They have been the main food source for the Mbuti pygmies of eastern Zaire (present Democratic Republic of Congo) (Milton 1985; Hart and Hart 1986), the Batek of Peninsular Malaysia (Endicott and Bellwood 1991), the Baka pygmies in the forests of southern Cameroon (Dounias 2001; Sato 2001), and people at Kuk Swamp of Papua New Guinea (Fullagar et al. 2006). The economically important species worldwide are *Dioscorea rotundata* (white guinea yam), *D. cayenensis* (yellow guinea yam), *D. dumetorum* (trifoliate or bitter yam), *D. alata* (yellow yam), *D. esculenta* (Chinese yam), *D. trifida* (cush-cush yam), and *D. bulbifera* (water or greater yam). Several wild species are also used as a source of food particularly during famines due to failure of staple food crops (Hahn 1995). About 30 species are grown on a minor scale for steroidal compounds such as sapogenin, dioscorin, and diosgenin for the pharmaceutical industry (Martin and Degras 1978; Orkwor 1998; Hahn 1995).

4.1.1 Origin and Distribution

The genus *Dioscorea* is considered to be among the most primitive of the Angiosperms and differentiated as Old and New world species (Coursey 1967; Hladik and Dounias 1993). The occurrence of *Dioscorea* spp. in southern Asia, Africa, and South America long predates human history, and domestication of the different species in these areas appears to have been by aboriginal man. The formation of the Atlantic ocean at the end of Cretaceous era seems to have separated the Old and New world species of yams (Coursey 1967). The desiccation of the Middle East during the Miocene period probably separated the African and Asian species (Coursey 1967). Yams are also believed to have originated in the tropical areas of three separate continents: Africa (mainly West Africa for *D. rotundata*, *D. cayenensis* and *D. dumetorum*), Southeast Asia (for *D. alata* and *D. esculenta*), and South America (for *D. trifida*). The Asiatic yam, *D. alata*, might have originated in tropical Burma

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and Thailand and *D. trifida*, the South American yam, is believed to date back to pre-Columbian times (Ayensu and Coursey 1972). Although *D. alata* reached the east coast of Africa at about 1500 BC from Malaysia, there is no evidence that it played any significant role in the evolution of cultivated African yams.

In West Africa, domestication of yam started as early as 50000 BC, during the Paleolithic era (Davies 1967). However, archeologists believe that actual cultivation of yam started about 3000 BC, about the same time when it started in Southeast Asia (Coursey 1967; Davies 1967; Alexander and Coursey 1969). The earliest domesticated yams in West and Central Africa are *D. rotundata*, *D. cayenensis,* and *D. dumetorum*, while in Southeast Asia, it was *D. alata* that was first cultivated. *D. alata* moved to India and Pacific Ocean more than 2,000 years ago (Coursey and Martin 1970). It is believed that there has been an east-to-west movement of yam species, wherein *D. alata* and *D. esculenta* moved westward to Africa and America, and the African species, *D. rotundata* and *D. cayenensis* moved westward to the Americas. The West African yam belt (Fig. 4.1) comprising Nigeria, Republic of Benin, Togo, Ghana, Cameroon, and Côte d'Ivoire is believed to have the oldest yam culture and constitute the largest yam biodiversity. About 90% of world yam is cultivated in this belt with *D. rotundata* and *D. cayenensis* accounting for most of the production. They are mostly preferred in West Africa owing to their organoleptic properties of the tubers but *D. alata* has the widest geographical distribution among the food yams (Martin 1976).

Currently yams are cultivated in about 50 tropical countries on 4.6 million ha worldwide with an annual production of about 52 million tons (FAO 2007). However, not all the countries (such as China) are recorded under Food and Agriculture Organization (FAO) statistics. More than 96% of world supply of fresh yam tubers comes from Africa, while four countries in West Africa namely Nigeria (72%), Cote d'Ivoire (9.5%), Ghana (6.6%), and the Republic of Benin (4.3%) account for about 92% of this output with 48.5 million tons of fresh tuber production per year (FAO 2007; Table 4.1). Most of the production in these regions comes from *D. rotundata* with the exception of Cote d'Ivoire, where *D. alata* accounts for 70% of yam production (Doumbia 1998), although 75% of domestic yam trade involves *D. rotundata* (Touré et al. 2003). World production of yams is reported to have tripled between the periods 1961–1963 and 1994–1996 (Lev and Shriver 1998). This has been attributed principally to increases in area of cultivation although yield increases were also recorded. In West Africa in general, and in Nigeria in particular, the increase in area planted with yams corresponds to an expansion of yam cultivation from the humid forest to more favorable conditions in the moist savanna (Manyong et al. 1996).

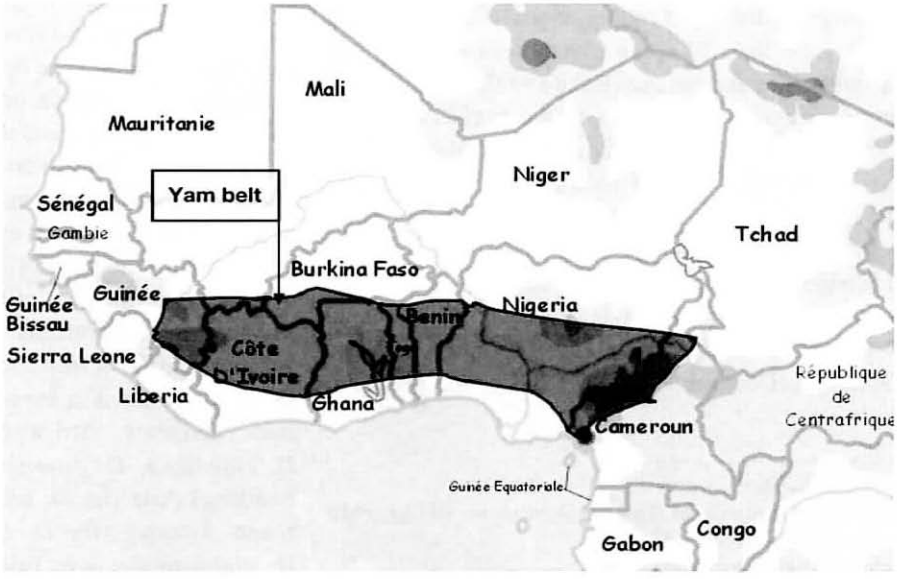

Fig. 4.1 The West African Yam belt. Source: http://www.cirad.org

Table 4.1 Area, production, and productivity of yam

Regions/Countries	Area (m ha)	Fresh tuber production (m tons)	Average yield (t/ha)
World	**4.6**	**51.5**	11.2
Africa	**4.4**	**50.1**	11.4
East Africa	0.04	0.25	7.0
North Africa	0.06	0.14	2.3
Middle Africa	0.1	1.1	6.8
West Africa	4.2	48.5	11.5
Benin	0.2	2.2	11.4
Burkina Faso	0.003	0.02	6.7
Cote d'Ivoire	0.5	4.9	9.8
Ghana	0.3	3.4	11.9
Guinea	0.004	0.04	10.9
Liberia	0.002	0.02	8.3
Mali	0.003	0.07	22.6
Mauritania	0.0004	0.003	6.3
Nigeria	3.1	37.2	12.1
Togo	0.06	0.6	10.0
Asia	**0.02**	**0.2**	15.0
East Asia	0.01	0.17	21.6
Southeast Asia	0.01	0.03	5.1
America	**0.2**	**1.2**	8.0
Central America	0.06	0.07	6.0
Caribbean	0.07	0.5	6.3
South America	0.07	0.6	10.2

Source: http://www.faostat.org

4.1.2 Importance of Yams

Species in the genus *Dioscorea* is extremely widespread in most of the tropical and subtropical regions. They are principally grown for food and have organoleptic qualities that make them the preferred carbohydrate food where they are grown. However, their storage organs (underground and/or aerial tubers) are also sources of proteins, fats, and vitamins for millions of people in West Africa. In countries where yams are generally cultivated, wild yams are used as food in times of shortage or famine (Coursey 1967; Fig. 4.2). The wild forms of *D. dumetorum* along with other species such as, *Dioscorea praehensilis*, *D. latifolia*, *D. preussii*, and *D. smilacifolia* are used as food in emergency throughout West Africa (Dalziel 1937; Labouret 1937; Irvine 1952). Similarly, in East and Central Africa, a number of wild species including *D. sansibarensis*, *D. preusii*, *D. cochleari-apiculata*, *D. schimperiana*, and *D. minutiflora* are used as famine food (Burkill 1939; Walker 1952). In African tropical rain forests, *D. praehensilis*, *D. mangenotiana*, *Dioscorea burkilliana*, and *D. semperflorens* are used as food (Sato 2001). The Asiatic species, *D. hispida*, which is closely related to *D. dumetorum*, is also used as food when there is shortage in parts of India and China (Burkill 1939). Certain wild species, such as, *D. sylvatica* Ecklon, are sold in the markets in Zimbabwe for treating skin diseases and chitsinga (physical disorder characterized by pain and swelling of the joints) (Gelfand et al. 1985). These wild species, although consumed only under famine conditions, also makes enormous contribution to human welfare. Apart from food, *Dioscorea* species are also used in pharmaceutical industries as sources of biologically active compounds or their precursors. Important but neglected species such as *D. villosa*, *D. praehensilis*, and *D. togoenensis* are known to have medicinal properties. *D. villosa*, for instance, is believed to benefit the liver and endocrine system. It regulates the female reproductive system, particularly during menstrual distress and menopause, and is also used in treating infertility. It is an effective treatment for morning sickness when used with chaste berry and dandelion. It is also famed

Fig. 4.2 Wild *Dioscorea* plants and tubers, mostly used as food. Photos by BJ Park and H Kikuno

for its steroid-like saponins, which can be chemically converted to progesterone contraceptives and cortisone. Similarly, *D. praehensilis*, variously referred to as Bush Yam or forest yam, has bitter tuber, which can only be eaten after careful preparation. Its young shoots are eaten in Bas Congo (DR Congo). In Gabon, the tuber is only eaten when young and after long cooking, all aimed at detoxifying it. In northern Nigeria, it is eaten as famine food.

Madagascar is very unique in both numbers of species that exists and their uniqueness in forms. They are unique in three ways (1) in their degree of endemism (most belong to an endemic clade, which represents one of the main lineages within *Dioscorea*); (2) almost all species have edible tubers; and (3) they are extracted from forest as wild plants. Studies have shown that there exists about 40 species of *Dioscorea* in this region, with 32 of them endemic to this region (Wilkin et al. 2007). Some of these species are *D. alatipes*, *D. arcuatinervis*, *D. bemarivensis*, *D. hexagona*, *D. karatana*, *D. maciba*, *D. namorokensis*, *D. ovinala*, *D. proteiformis*, and *D. ambrensis* (documented and conserved at Royal Botanic Gardens, Kew, UK). These species are very important to Malagasy people, especially on a local scale, as food providers or as medicines derived from the forests or from small-scale cultivation. The tubers are used as a starch source and can be eaten raw (*D. soso*, *D. fandra*), others are simply boiled or baked (*D. nako*), while some need extensive preparation (*D. antaly*). Traditional medicinal uses are a feature of *Dioscorea*, since the genus is rich in steroidal saponins. The most frequently encountered medicinal use of yams in Madagascar is the treatment of burns, ulcers, and other skin complaints with the bulbils of *D. bulbifera*.

Despite their economic and socio-cultural importance, there is limited knowledge about the origin, phylogeny, diversity, and genetics of these wild yams due to research neglect and several biological constraints (Mignouna et al. 2007). These wild species may serve as an important source of genetic variation in yam breeding work especially for resistance to pests and diseases. Further genetic improvement to reduce the bitter constituents in some of the species may render them more palatable and popular. It is therefore imperative to clarify the cytogenetic status, e.g., chromosome number of wild yams to enhance their usage in future breeding work.

4.1.3 Domestication of Yams in West Africa

The domestication of wild yams is a common practice mainly in West Africa that offers an insight into how farmers tap wild genetic resources to create products suitable for agriculture. However, until recently, breeders or agronomists have not focused enough attention to understand such an organized process of generating on-farm biodiversity through introduction of relatively new material that could be exploited through participatory plant breeding (involving farmers and breeders together). It is believed that farmers collect the tubers of wild yams (or natural interspecific hybrids) from forest areas during hunting and brought under cultivation with intense vegetative multiplication and selection procedure (at different periods of time making it a lengthy procedure) that induced changes in plant characteristics (both morphological and biochemical changes), mainly in tuber characteristics, making it a completely different variety (Mignouna and Dansi 2003). However, only limited research has been done to understand this process of domestication followed by these farmers to generate agricultural biodiversity. In West Africa, yam is basically subjected to monocropping followed by societies that practice something called "civilization of the yam" (Miège 1952). These societies are highly organized and well structured with their food needs mainly covered through production from *D. rotundata*. Burkill (1939) was convinced that *D. rotundata* resulted from the process of domestication that African farmers practiced to bring wild forms into agriculture. This hypothesis was not clear until recently when numerous studies using powerful tools (such as enzymatic and molecular markers; flow cytometry) were carried out to understand the relationship between *D. rotundata* and wild yams. In addition, social surveys carried out in Benin (Dumont and Vernier 1997) and Nigeria (Vernier et al. 2003) showed similar practices of domestication by farmers in these regions. Hildebrand (2003) also reported a similar kind of domestication process in southwestern Ethiopia where several wild yams have been brought under cultivation. In addition, Baco et al. (2007) hypothesized that high level of diversity of yam varieties exist in West Africa and this is more related to farmers' ethnic group. This practice differs from one ethnic group to another within a given area, but remains constant for a given ethnic group independent of its geographical location.

In the yam belt of West Africa, the situation is more confusing with the occurrence of a species complex, *D. rotundata* Poir. and *D. cayenensis* Lam., also known as Guinea yams. These two species are phenotypically distinguishable with *D. rotundata* (white yam) having white-fleshed tubers and 6–8 months growth period, and *D. cayenensis* (yellow yam) with yellow-fleshed tubers and 8–12 months growth period. However, the descriptions of both Lamarck (1792) and Poiret (1813) seem inadequate to separate these two species clearly (Miège and Lyonga 1982). In 1936, Chevalier created a new subsection, Cayenenses, under the section Enantiophyllum to include Guinea yams and all their wild relatives. However, the studies based on morphological characters are not conclusive enough to distinguish these two species clearly and the debate continues. Miege regarded *D. rotundata* as a subspecies of *D. cayenensis* in his book *Flora of West Tropical Africa* (1968 edition). Hamon (1987) pooled all West African cultivated yams that are not bulbiferous under this species complex. *D. rotundata* and *D. cayenensis* were domesticated from plants belonging to wild Dioscoraceae of the Enantiophyllum section (Burkill 1939; Miège 1952; Hamon 1987; Terauchi et al. 1992).

D. cayenensis is found in West and Central Africa. In West Africa, it coexists with *D. rotundata* but not widely cultivated, while it is grown along with *D. alata* in most of the forest areas in central Africa where *D. rotundata* is rather limited. Based on morphological characteristics, Miège (1982) proposed *Dioscorea abyssinica* Hochest ex. Knuth, *D. lecardii* De Wild., *D. liebrechtsiana* De Wild., *D. praehensilis* Benth. and *D. sagittifolia* Pax. as the possible wild progenitors of Guinea yams. Other wild yams that are morphologically related to Guinea yams are *D. burkilliana* J. Miege, *D. mangenotiana* J. Miege, *D. minutiflora* Engl., *D. smilacifolia* De Wild. and *D. togoensis* Knuth (Chevalier 1936; Miège 1982). Of all these species, *D. burkilliana*, *D. liebrechtsiana*, *D. minutiflora*, *D. mangenotiana*, *D. smilacifolia*, and *D. praehensilis* are found in rain forests, while the rest are found in the Savannas. Within each ecological zone, these wild species are distributed widely without any geographical isolation. Studies were further conducted using molecular markers to find a solution to this controversy. Dansi et al. (2000a) reported that

isozymes (leaf proteins) could differentiate the accessions of the two species, supporting the idea that *D. rotundata* and *D. cayenensis* are two distinct species. Studies conducted using chloroplast DNA showed that *D. rotundata* and *D. cayenensis* bear the same chloroplast genome, type A (which should make them the same species), as three other wild species *D. praehensilis*, *D. liebrechtsiana*, and *D. abyssinica* (Terauchi et al. 1992; Ramser et al. 1997; Chaïr et al. 2005). Based on nuclear ribosomal DNA, Terauchi et al. (1992) suggested that *D. cayenensis* is an interspecific hybrid with male parent being either *D. burkilliana*, *D. minutiflora*, or *D. smilacifolia* and the female parent being either *D. rotundata*, *D. abyssinica*, *D. liebrechtsiana*, or *D. praehensilis*. Mignouna et al. (2004) used the PCR-based marker, random amplified length polymorphism (RAPD), to establish the relationship between wild and cultivated yams, and showed that the accessions of *D. rotundata* could be clearly separated from the accessions of *D. cayenensis*. The accessions of *D. rotundata* showed a higher degree of polymorphism and were more closely related to *D. praehensilis* and *D. liebrechtsiana*.

Several researchers have suggested the phylogenetic proximity of *D. cayenensis* to *D. burkilliana* (Akoroda and Chheda 1983; Onyilagha and Lowe 1985; Mignouna et al. 1998; Dansi et al. 2000b). There are several morphological characteristics that support this suggestion. Hamon (1987) reported that the tubers of both the species appear very similar when grown in Cote d'Ivoire. In Cameroon too, *D. cayenensis* tubers have been reported to appear similar to those of *D. burkilliana* (Dumont et al. 1994). However, various arguments have been put forward regarding the morphological variability in tuber characteristics of *D. cayenensis* observed throughout West and Central Africa, suggesting that *D. cayenensis* probably has multiple origins or may have originated from two probable ancestors, *D. burkilliana* or *D. minutiflora*. In other studies, *D. minutiflora* has been considered as a form of *D. burkilliana* (Mignouna and Dansi 2003; Chaïr et al. 2005), indicating that the species is highly polymorphic. Hamon (1987) described the existence of two genetic forms of *D. minutiflora* using isozymes. The debate continues and the polyploid nature of *D. cayenensis* makes it more difficult to make a clear conclusion about the kinship of this species with *D. burkilliana* and *D. rotundata*, indicating the need for further investigation.

4.1.4 Germplasm Collection and Conservation

Yam has great economic and social significance in sub-Saharan Africa representing greatest genetic diversity of this crop in this region. The diversity under cultivation is further enhanced by the ongoing domestication of wild yam in various countries (Mignouna and Dansi 2003; Scarcelli et al. 2006). Although many authors suggested that most of the pre-domesticated yams are wild because they were collected from non-cultivated areas (Hamon et al. 1992; Dumont and Vernier 2000; Mignouna and Dansi 2003; Tostain et al. 2003), it has recently been concluded that a constant gene flow occurs between the cultivated species complex (*D. cayenensis–D. rotundata*) and its wild related species making it difficult to clearly identify the varieties as either wild or cultivated.

Wild yams are sexually propagated while cultivated yams are vegetatively propagated; however, farmers carry out intense vegetative propagation of the plants collected from forest areas (which could be wild or interspecific hybrids) for long periods of time, contributing to the domestication of species. Furthermore, yam domestication has been a factor for the degradation of forests and fertile lands, and also contributing to the loss of landraces. Therefore, it is of paramount importance to invest in yam conservation for food security and preservation of genetic diversity in the tropical and subtropical areas.

There are two approaches for plant genetic resources conservation, namely, (1) in situ conservation that involves maintaining plants in the area where they developed their distinctive properties, i.e., in the wild or in farmer's field. This is certainly the optimal option as it allows germplasm to evolve with its natural environment. (2) Ex situ conservation that involves conservation of full plants or propagules out of their natural environment and includes seedbank, field bank, arboretum, botanical gardens, etc. The International Institute of Tropical Agriculture (IITA), Ibadan, Nigeria, maintains the largest world collection of yams, accounting for over 3,087 accessions of West African origin. Eight species are represented in the collection comprising accessions of *D. rotundata*, *D. alata*, *D. dumetorum*, *D. cayenensis*, *D. bulbifera*, *D. mangenotiana*, *D. esculenta*, and *D. preusii*. Two

species, *D. rotundata* and *D. alata* account for 67% and 25% of the total collection, respectively. These accessions are held in trust at Food and Agricultural Organization (FAO) and are distributed without restriction for use in research for food and agriculture. Other collections have been reported in Africa (Burkina Faso, Cameroun, South Africa and Uganda); West Indies (Barbados, Cuba, Guadeloupe, Jamaica, Saint Dominique, Trinidad-Tobago); America (Brazil, Colombia, Costa rica, Guatemala, Mexico, Panama, USA); Pacific (Cook Islands, Fiji, Niue Island, New Caledonia, Papua New Guinea, Solomon Islands, Tonga, Vanuatu, western Samoa); and Asia (Bangladesh, India, Indonesia, Japan, Nepal, Malaysia, Philippines, Sri Lanka, Thailand, Vietnam) (Malaurie 1998; Lebot 2009). In addition, the Global Crop Diversity Trust (http://www.croptrust.org) is presently supporting the regeneration of several national collections in West Africa such as in Benin, Togo, Ghana, and Cote d'Ivoire.

The easiest and cheapest ex situ conservation strategy involves collection of germplasm accessions in the form of seeds to produce orthodox seeds. Several yam species have been reported to produce such seeds (Daniel 1997). However, the varieties with the most desirable traits quite often do not flower (Lebot 2009) and hence do not produce seeds. Because of this, traditional yam conservation is achieved via the establishment of field banks. At IITA, a field gene bank has been established wherein yam germplasm is vegetatively propagated by planting setts from underground or aerial tubers. Plants are grown in the field for about 6–9 months, depending on the species or genotype. Mature tubers are then dug up or aerial tubers are plucked and stored in a traditional yam barn under ambient temperature or at 15°C for 4–5 months. However, there is a high risk of genetic erosion in the field and in storage due to occurrence of diseases and attack of pests, adverse climatic conditions and also likeliness of theft. In addition, maintenance of field banks are expensive and laborious. In vitro conservation offers an alternate approach for ex situ conservation of yam. There are added advantages as it reduces the risk of germplasm loss due to above listed factors and allows maintenance of disease-free germplasm in a limited space under pest and pathogen-free condition, and facilitates safe exchange of germplasm at the international level (Hanson 1986; Ng 1988). In vitro conservation is followed in *Dioscorea* spp. through nodal cutting or meristem culture. However, the combination of optimal mineral and growth regulators varies depending on species and genotypes (Mantell et al. 1978; Saleil et al. 1990; Malaurie et al. 1995a, b; Sedigeh et al. 1998; Myouda et al. 2005). Malaurie et al. (1993) established an in vitro germplasm collection for 16 *Dioscorea* species from Africa and Asia. The collection consisted of ten wild species (*D. abyssinica, D. bulbifera, D. burkilliana, D. dumetorum, D. hirtiflora, D. mangenotiana, D. minutiflora, D. praehensilis, D. schinzperana, and D. togoensis*), five edible species (*D. alata, D. bulbifera, D. cayenensis–D. rotundata* complex, *D. dumetorum*, and *D. esculenta*), and one interspecific hybrid (*D. cayenensis–D. rotundata* complex, cv. Krengle × *D. praehensilis*). Similarly, an in vitro genebank has been established at IITA that conserves over 1,500 accessions of yam wherein 5–10 seedlings of each accession are stored at 16 ± 2°C under a 12 h photoperiod (Dumet et al. 2007). Each seedling is either maintained in a test tube or a polyethylene bag on solid Murashige and Skoog-based medium (Dumet et al. 2007). Under such storage conditions, accessions are subcultured every 10–24 months depending on species and genotype.

In order to further rationalize ex situ conservation of plant tissues for long periods, several groups have investigated cryopreservation. Cryopreservation is conservation of plant tissues at very low temperature, generally using liquid nitrogen (−196°C) so that tissues could be stored in perpetuity. Two approaches are followed for cryopreservation of plant tissues. One is based on evaporative desiccation of plant tissue prior to freezing, which often involves encapsulation of tissues before dehydration treatment. Tolerance to natural or induced dehydration is the key factor for success of this method. The other approach involves the use of cryoprotectants such as DMSO (dimethyl sulphoxide), ethylene glycol, or glycerol. In addition to an osmo-dehydration effect, these compounds stabilize the plant tissues when submitted to freeze/thaw cycles. Successful cryopreservation has been reported for different species of yam such as *D. bulbifera, D. oppositifolia, D. alata, D. cayenensis, D. wallichii*, and *D. floribunda* (Mandal et al. 1996; Malaurie et al. 1998; Mandal 2000; Leunufna and Keller 2003). To date, there is no universal cryopreservation process for plant tissues, and in many cases, adjustments are made to suit a species or genotypes. Although cryopreservation is a promising approach for ex situ conservation, cryobank is yet to be

established for yam. It is assumed that in vitro storage, including cryopreservation, induced somaclonal variation, i.e., genotypic or/and phenotypic variations would be the best bet for long-term conservation in yams. At IITA, work is in progress to investigate integrity of cryopreserved germplasm.

Any ex situ germplasm collection (national, regional or international) is expected to capture maximum diversity and the knowledge on genetic diversity is obtained through proper characterization of germplasm collection using morphological descriptors or molecular markers for further utilization in crop improvement programs. At IITA, two types of databases are maintained for each accession. The passport data consist of information on a unique identifier for each genotype (accession number), its taxonomic data (genus, species, pedigree), geographical information (latitude, longitude, altitude), environmental data (market, farmer field, topography, soil type etc.), and collection data (collector's name, year of collection, collection number, etc.) (IPGRI/IITA 1997). The characterization data consist of information on agromorphological characters recorded following internationally standardized morphological descriptors for yam (IPGRI/IITA 1997). The passport and characterization data are maintained as databases and can be retrieved through online search using the URL http://genebank.iita.org/search. A core collection of yam (391 accessions) has also been established at IITA representing 75% of genetic diversity. Data on 99 morphological descriptors were used to stratify the global collection based on species and country of origin to define the core collection (Mahalakshmi et al. 2006). However, the analysis did not take into consideration the sex and ploidy status of the accessions, two attributes important to breeders who may want to use the core accessions in yam improvement programs. The passport or characterization data of most of the accessions are also incomplete and duplicates are yet to be eliminated. Yam germplasm has been characterized for morphological characters (Dansi et al. 1998, 1999, 2000a, b), physico-chemical characteristics (Lebot et al. 2006), organoleptic properties (Egesi et al. 2003), soluble tuber protein profiles (Ikediobi and Igboanusi 1983), or isozyme patterns (Lebot et al. 1998; Dansi et al. 2000a; Mignouna et al. 2002a). Similarly, use of molecular markers such as amplified fragment length polymorphism (AFLP), RAPD and simple sequence repeat (SSR) markers have also been reported (Mignouna et al. 2003; Malapa et al. 2005; Egesi et al. 2006; Scarcelli et al. 2006; Tamiru et al. 2007; Tostain et al. 2007) in diversity studies of yams. Efforts are underway at IITA to use molecular markers, such as SSRs, to fingerprint the entire collection, mainly to identify the duplicates in the collection and establish a core collection using information on morphological and molecular data.

Another important aspect of conservation strategy is the distribution of germplasm to various users. In case of yam, the distribution is driven by the International Treaty on Plant Genetic Resources for Food and Agriculture (ITPGRFA), an international agreement with the overall goal of supporting food security via germplasm exchange and benefit sharing. At IITA, germplasm exchange is carried out following the phytosanitary procedures during which germplasm is rendered free of regulated quarantine pests and pathogens. Efforts are also being made to establish virus-free germplasm through meriseming and selection of virus-free germplasm through virus indexing.

4.1.5 Botany

The word "Yam" is applied only to members of the genus *Dioscorea* (Alexander and Coursey 1969) that belong to family Dioscoreaceae in the order Dioscoreales classified under the monocotyledons. Most of the wild species of African origin are included within this section. The order Dioscoreales has been redefined and now comprises three families: Burmanniaceae, Dioscoreaceae, and Nartheciaceae (Chase et al. 1995; Caddick et al. 2000, 2002). The genus *Dioscorea* is the largest genus within Dioscoraceae comprising about 600–850 species (Knuth 1930; Burkill 1960), although Ayensu (1972) estimated that there are less than 200 species that are distributed across the tropics and subtropics. Currently, the world checklist in Royal Botanic Gardens, Kew includes 644 accepted species in Dioscoreaceae in five genera, which are *Dioscorea*, *Rajania*, *Tacca*, *Stenomeris*, and *Trichopus* (Govaerts et al. 2007). In a taxonomic status based on gross morphological characters, the genus *Dioscorea* is divided into five sections: Enantiophyllum, Combilium, Opsophyton, Macrogynodium, and Lasiophyton (Burkill 1960). The most important edible yam

species – *D. rotundanta*, *D. alata*, and *D. cayenensis* – and the minor economic species in temperate zone – *D. opposita* and *D. japonica* – belong to the section Enantiophyllum. *D. dumetorum*, *D. hispida*, and *D. pentaphylla* belong to the section Lasiophyton; *D. bulbifera* to the section Opsohyton; *D. esculenta* to the section Combilium; and *D. trifida* to the section Macrogynodium.

The yams are Angiosperms, or flowering plants, and are twining climbers, and produce dry capsules. All species of economic importance are tuberous, sometimes producing aerial tubers called bulbils. Majority of *Dioscorea* species are distributed throughout the tropics, while a few economically important species are also found in the warmer regions of the temperate zones (Bai and Ekanayake 1998). Wild species are either annuals (with aerial and underground tubers growing annually) or semi-perennials (aerial part growing in 12–24 months cycle, along with perennial underground part) or perennials (aerial and underground parts growing over several years). Cultivated species are generally grown as annuals. There are huge differences in size, shape, and number of tubers per plant within and between species.

The tuber of yams is a storage organ, which forms a new tuber and shrivels away simultaneously when the regrowth is induced. As the organ lacks the typical characteristics of a modified stem structure, the tuber has no pre-formed buds or eyes, no scale leaves, and no terminal bud at the distal end of the tuber (Hahn et al. 1987). Some perennial species produce tubers that become larger and more lignified as the plant ages. Some species, such as *D. bulbifera*, *D. alata*, *D. opposita*, and *D. japonica* produce bulbils in leaf axils on vine of matured plant in addition to underground tubers. The Enantiophyllum species usually produce one to three large tubers, while *D. esculenta* (Combilium) produce 5–20 tubers; *D. dumetorum* (Lasiophyton) produces 3–12 tubers and *D. trifida* (Macrogynodium) produces a larger number of tubers that are small in size. The number and shape of yam tubers vary depending on the species and genotype (Martin and Sadik 1977; Okonkwo 1985).

The yam plant possesses shallow fibrous root system that is normally unbranched and concentrated within the top 0.3 m of the soil and very few actually penetrate up to 1 m depth (Onwueme 1978). Several roots rapidly develop and reach 3–4 m in radius around the plant after sprouting. According to Onwueme (1978), yam possesses three different types of adventitious roots (1) adventitious roots arising from the corm-like structure at the base of the stem, whose function is to absorb minerals, nutrients, and water from the soil; (2) adventitious roots arising from the body of the yam tubers; and, (3) adventitious roots originating from the exposed lower nodes of the growing yam plants. Fibrous roots are smooth in general but some species have stems and roots with spines. The wild relatives of yams have more spiny roots than the cultivated species (Onwueme 1978).

The stems of all *Dioscorea* species, except for few, climb by twining, and the direction of twining of the vine, i.e., anticlockwise or clockwise, is a characteristic peculiar to the particular section within the genus *Dioscorea*. Species of the Enantiopyllum section twine to the right (clockwise) and those of the Combilium, Opsophyton, Macrogynodium, and Lasiophyton sections twine to the left (Onwueme 1978). In many species, for instance, *D. cayenensis*, *D. esculenta*, and *D. nummularia*, vines have spines which support the twining habit while also deterring animal predators (Okonkwo 1985). The wings present in some species, such as *D. alata* and *D. colocasiifolia*, also support the twining habit. The length of stems generally varies depending on the species. *D. esculenta* rarely climbs more than 2–3 m while some forms of *D. rotundata* may climb to 10–12 m under favorable conditions (Coursey 1967). Some wild species, such as, *D. sansibarensis*, *D. preussii*, and *D. mangenotiana* may even be longer.

Most of *Dioscorea* species have simple, cordate, or acuminate leaves borne on long petioles but in some species, they could be lobed or palmate with pointed tips. *D. trifida* has lobed leaves consisting of three leaflets while *D. dumetorum* has compound leaves (Okonkwo 1985). In general, the leaves vary in shape, size, and color (pigmentation) from one species to another or even between individuals of same species. Yam leaf lamina generally has three primary nerves joining at the tip of the lamina. The area of lamina in cultivated species is about 50–200 cm^2, although in some wild species such as, *D. sansibarensis*, it may be much larger. The leaf arrangement on the stems is usually described as opposite or alternate depending on species or growth of the stem, i.e., alternate on the lower part of the stem and opposite on the upper (younger) part (Onwueme 1978). The leaf petiole is long and depending on species could be

winged or spined. Both stems and leaves of many *Dioscorea* species are covered with hair.

The flowers of yam are basically dioecious, with male (staminate) and female (pistillate) flowers borne separately or on separate plants. In some cases, monoecious plants with staminate and pistillate flowers are occasionally observed among the genotypes of *D. rotundata* (Sadik and Okereke 1975a, b). Generally, the female plants are less in number with fewer flowers than male plants. The male or female flowers are borne on axillary spikes in the leaf axils of yam vines. Flowering in the major edible yams has been reported to be sparse, irregular, or absent. The male flowers are sessile, glabrous, and spherical and are borne axially or terminally. These flowers consist of a calyx of three sepals and a corolla of three petals, arranged regularly and almost similar in size and appearance, with three or six stamens (Onwueme 1978). The ovary of female flowers is trilocular with each locule having 2–3 ovules and is located below the corolla (Miége 1968; Sadik and Okereke 1975a, b). The flowers of all *Dioscorea* spp. are entomophillous and are pollinated mainly by insects (Coursey 1967; Fig. 4.3). It is assumed that the sweet scent of the flowers mainly attract these insects, although the species involved have not yet been identified (Govaerts et al. 2007).

The flowers are succeeded with the formation of dry, dehiscent, trilocular capsules (1–3 cm long) with each fruit producing six seeds. The seed in each capsule is small and has wings that vary in shape in different species (Coursey 1967; Lawton 1967; Onwueme 1978). The seeds are flat and light and the wings help in wind dispersion. The seed germination process is well explained by Lebot (2009).

Some *Dioscorea* spp. such as *D. alata*, *D. bulbifera*, *D. pentaphylla*, *D. opposita*, and *D. japonica* produce bulbils in the axils of leaves. They are specifically adapted for vegetative propagation and have the appearance and morphology of condensed stems (Coursey 1967). They are smaller than the underground tubers, but in *D. bulbifera*, these are the main storage organ and are larger in size. Bulbils can be toxic in some species, while in others they are fine-textured and are appreciated for taste (*D. bulbifera*). Short day length generally accelerates formation of bulbils.

4.1.6 Constraints to Yams

Low soil fertility, weed competition, pests, and diseases in the field (foliar and soil borne) and in storage, and attack by animals (pigs and rodents) limit the production and productivity of yam cultivation wherever they are grown (Kenyon et al. 2003; Baimey et al. 2006; Coyne et al. 2006; Tchabi et al. 2008; Lebot 2009). These factors, and intensive cultivation of improved cultivars, are the factors responsible for genetic erosion of wild yams and landraces. Although most of the documented information is related to the cultivated species, in general, the information on the economic significance of pests and diseases under farmers' conditions is often lacking or inadequate although yield losses of up to 100% have been attributed to them in experimental trials (Lebot 2009). The economic importance of insect pests (e.g., leaf and tuber beetles, mealy bugs and scales), fungal diseases (e.g., anthracnose, leaf spot, leaf blight and tuber rots), and viral diseases (caused by poty- and badnaviruses), as well as nematodes also vary depending on the agro-ecological zone, cropping system, and production practices. For instance, anthracnose disease caused by *Colletotrichum gloeosporiodes*, mosaic disease caused by yam infecting poty- and badnaviruses, root rot nematodes (*Meloidogyne* spp.), tuber rot, and scale insects are considered as most economically important pests and diseases in West African yam belt (Kenyon et al. 2003; Coyne et al. 2006). Integrated pest management and crop improvement are being pursued at IITA to manage these constraints in West Africa.

Availability of "clean" planting material is the greatest limitation in West Africa. Farmers in West Africa bulk planting material (tubers, veins and setts) from both infected (symptomatic) and asymptomatic plants and use them indiscriminately for planting. This practice not only contributes to the spread of pests and pathogens along with the planting material but also affects the yields and gradual decline in source material. Efforts are being made to harmonize clean seed yam systems in West Africa which would improve the quality of farmer-preferred materials. A similar system needs to be conceived to conserve the wild yams and landraces and protect them from pests, diseases, and soil erosion.

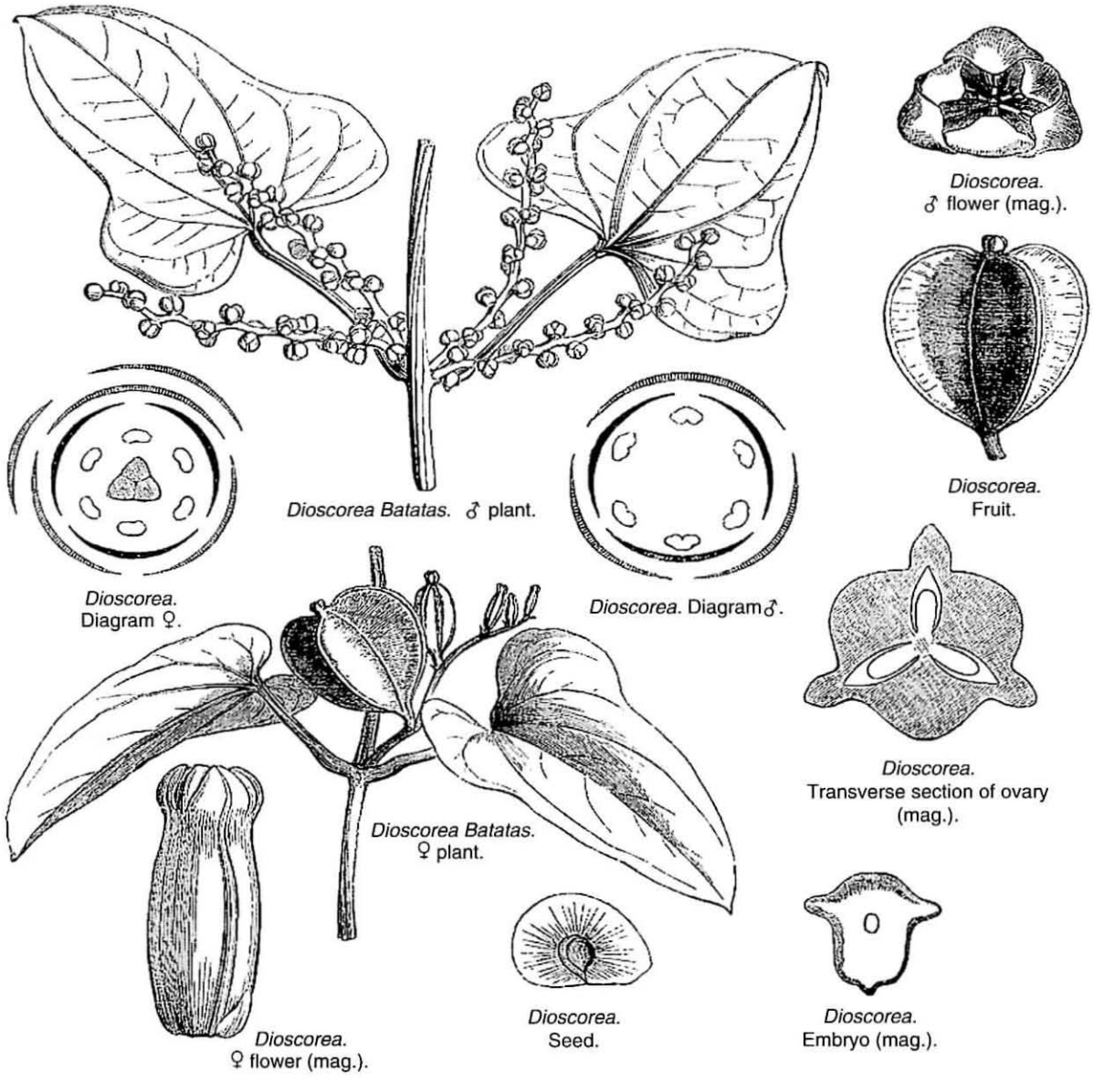

Fig. 4.3 The plant, male and female flower, embryo, and fruit of a wild *Dioscorea* species. Source: Watson and Dallwitz (1992)

4.1.7 Cytogenetics

Dioscorea is one of the most difficult genera for cytotaxonomic and cytogenetic studies (Essad 1984) because of small size of the chromosomes and flowering in some species, hence chromosome counts of only about 55 species have been reported so far (Essad 1984; Degras 1993). However, the genus offers an attractive model for the investigation of ploidy events and chromosome evolution in wild and cultivated species in relation to their dioecious nature, vegetative/sexual propagation, and the process of domestication (Bousalem et al. 2006). The existence of various ploidy levels and the lack of a diploid relative to the cultivated polyploid yam leads to various complications in cytogenetic studies in yams. Observations have been restricted in most cases to the determination of chromosome numbers and chromosome pairing

from mitotic (Sharma and De 1956; Raghavan 1958, 1959; Ramachandran 1968; Essad 1984) and meiotic cells (Abraham and Nair 1990; Abraham 1998). Many authors have reported the difficulties encountered in chromosome counting in cultivars of *D. cayenensis/ D. rotundata* complex (Miège 1954; Baquar 1980) and other *Dioscorea* species (Ramachandran 1968; Baquar 1980; Essad 1984; Gamiette et al. 1999).

The basic chromosome number of yam species is controversial, although it has been acknowledged as being $x = 10$ or $x = 9$, with a high frequency of polyploid species. Tetraploid species are the most frequent, followed by diploids ($2x$), hexaploids ($6x$), and octaploids ($8x$) (Essad 1984). The base chromosome number $x = 10$ (found in all Asian species) is present in only 52% of the African species and 13% of the American species examined so far (Bousalem et al. 2006). Recent data have revealed a basic chromosome number of $x = 20$ (Scarcelli et al. 2005), but this is based on a small number of yam species. The highest chromosome number and the smallest chromosome sizes are reported to occur more in tropical *Dioscorea* species, while the smallest number and largest sizes occur more in temperate species. This coupled with the fact that two new basic chromosome numbers $x = 6$ (Segarra-Moragues and Catalán 2003; Segarra-Moragues et al. 2004) and $x = 20$ (Scarcelli et al. 2005) have also been found in recent years, suggest that more work will be needed to understand the cytogenetics of both cultivated and wild yams. Furthermore, it is important for yam breeders to determine the ploidy status of clones, especially of new introductions before they can be utilized in a breeding program (Egesi et al. 2002). These fundamental data are, therefore, essential for sexual breeding of yams, which will enable ploidy manipulations in intraspecific crosses, effective breeding and conservation of the species, and for elucidating the phylogeny and the origins of the yam and the evolution of the genus *Dioscorea*. For this purpose, flow cytometry has been recently used to determine ploidy levels in yams (Gamiette et al. 1999). The method is non-destructive (one sample can be prepared from a few milligrams of leaf tissues), exceptionally rapid, sensitive and convenient, does not require dividing cells, and can detect both mixoploidy and aneuploidy (Galbraith et al. 1983; De Laat et al. 1987; Arumuganathan and Earle 1991a, b; McMurphy and Rayburn 1991; Dolezel 1997). Ploidy determination of *D. alata*, *D. cayensis-rotundata* and some wild yam species by flow cytometry and conventional chromosomes counting revealed variable ploidy levels of $4x$, $6x$, and $8x$ in the species (Gamiette et al. 1999) with majority being tetraploid (Dansi et al. 2000a). Hamon et al. (1992) showed a continuous range of ploidy ($3x$, $3.5x$, $4.5x$, $6x$, $7x$, $8.5x$) in *D. alata* by flow cytometry, with occurrence of high proportion of hexaploids (Egesi et al. 2002). Other studies have classified *D. rotundata* as diploid with a basic chromosome number of 20 (Daïnou et al. 2002; Scarcelli et al. 2005), but evidence of Scarcelli et al. (2005) is based on progenies from a monoecios plant rather than dioecious plants.

The inheritance of ploidy in yams, that is, allo- or, autoploid, is also not clear. More studies are necessary to identify the inheritance patterns of the polyploid yams. Identification based on multivalent formation will be misleading since autotetraploid species do not always exhibit multivalent formation (Soltis and Riesberg 1986) and allotetraploid species have been shown to form multivalents at times (Watson et al. 1991). Therefore, analyses of segregating populations have been extensively used to assess inheritance patterns (Demarly 1958; Jackson and Casey 1982). Recently, segregation analysis using isozyme and SSR markers led to the conclusion that *D. rotundata* ($2n = 4x = 40$) is a diploid species (Scarcelli et al. 2005), while that based on AFLP markers reflected a disomic inheritance in *D. alata* and *D. rotundata* (Mignouna et al. 2002b, c), revealing an allotetraploid structure for the two species. Segarra-Moragues et al. (2004) also concluded on the basis of SSR patterns that the wild species, *D. pyrenees* and *D. chouardii*, are allotetraploid. SSR segregation analysis and cytogenetic evidence revealed a tetrasomic behavior and an autotetraploid structure of the genome of the American species, *D. trifida* (Bousalem et al. 2006). The situation in other *Dioscorea* species could also have a base chromosome number of $x = 20$ rather than $x = 10$ and most of them could be di-, tri- or tetraploid in nature. It is confirmed that polyploidy is common among *Dioscorea* species and accessions having 40 chromosomes are most common, followed by accessions with 20, 60, and 80 chromosomes (Lebot 2009). There are reports of accessions having 100 chromosomes (*D. bulbifera* and *D. esculenta*), 120 chromosomes (*D. hastata*, *D. minutifolia*, and *D. smilacifolia*), and 140 chromosomes (*D. opposita*, *D. pentaphylla* and *D. cayenensis*).

Further studies based on genomic in situ hybridization (GISH) and florescent in situ hybridization (FISH) will probably provide better information on the inheritance of ploidy in yams. These techniques can be used to localize the presence or absence of specific mRNA or DNA sequences on chromosomes.

4.1.7.1 Relevance of Cytogenetics and Wild Yams to Cultivated Species

The cultivated yam species *D. rotundata* ($2n = 40$) has been considered as a tetraploid species with a basic chromosome number of ten (Scarcelli et al. 2005), suggesting that in diversity studies, one should anticipate the detection in individual genotypes of up to four alleles per DNA marker locus. Recent studies by various authors have challenged this assertion and the most parsimonious hypothesis from their work is to conclude that *D. rotundata* is a diploid (Zoundjihekpon et al. 1994; Daïnou et al. 2002; Mignouna et al. 2002a, b, c, d) since only two alleles have been commonly found in this species. Similar contentions have been observed in *D. alata* where Egesi et al. (2002) noticed that majority of plants used in their study were hexaploid (84.9%) with a smaller percentage of tetraploids (15.7%), contrary to the accepted theory that *D. alata* is a tetraploid. A higher number of male plants were also found to be hexaploid than tetraploids, again at variance with earlier findings, which reported that hexaploid male plants are rare. Higher ploidy levels were not directly related to sparse or erratic flowering as previously reported as profuse flowering occurred in some male hexaploid accessions (Egesi et al. 2002). Octoploidy, which is commonly noticed in both *D. rotundata* and *D. alata* (Hamon et al. 1992), or mixoploidy (Dansi et al. 2001) was not observed in this analysis. Similarly, there was no association of ploidy level to geographic origin of materials as was asserted by Miège (1954) and Essad (1984). Ploidy level in yam was shown to be now easy and rapidly determined using high-resolution flow cytometry (Egesi et al. 2002), making a case for revisiting the cytogenic information available especially in case of wild yams.

D. abyssinica and *D. praehensilis* are considered by Hamon et al. (1997) and Terauchi et al. (1992) as wild relatives of *D. rotundata*. These two species have been assumed to be tetraploid, with $2n = 40$ chromosomes (Miège 1952). It has been suggested that *D. praehensilis* is one of the parents of the cultivated *D. cayenensis* Lam., together with other species of the section *Enantiophyllum* such as *D. abyssinica* Hochst. ex Kunth and *D. burkilliana* J. Miège. It produces viable seeds even after long period of storage. The major challenge, therefore, is to trace the ploidy level of the cultivated species by looking at their ancestry, especially when Scarcelli et al. (2005) found that the cultivated species of *D. rotundata* and wild species, *D. abyssinica* and *D. praehensilis*, did not differ in number of alleles per locus.

4.2 Classical and Molecular Genetic Studies

The genetics of yams is least understood among the major staple food crops (Martin 1966; Zoundjihekpon et al. 1994) due to several biological constraints including, a long growth cycle (about 8 months or more), dioecy, poor- to non-flowering plants, polyploidy, vegetative propagation, heterozygous genetic background, and poor knowledge about the organization of the crop (Mignouna et al. 2007). In addition, although yams are monocots, they are very distantly related to grasses such as wheat, maize, rice, and sorghum, whose genomes are well studied. For example, banana and wheat are more closely related to each other than either is to yam. Hence there is no convenient model system for yam genomics and not much research effort has been made to understand the same. In recent years, some progress has been made in germplasm characterization and in the development of molecular markers for genome analysis in wild or cultivated yam species, and all the available information is presented in this section.

Yams have a relatively small genome size, estimated at 550 Mbp/1C for *D. alata* and 800 Mbp/1C for *D. rotundata* (Mignouna et al. 2002b, c). Molecular genetic analysis of the yam genome is gaining momentum. The initial effort in yam genomics was devoted to the development of polymorphic DNA markers and assessment of their potential application in yam. Framework genetic linkage maps based on amplified fragment length polymorphism (AFLP) markers have been constructed for *Dioscorea tokoro*, a wild relative of yam (Terauchi and Kahl 1999), and

for two cultivated species, *D. rotundata* (Mignouna et al. 2002b) and *D. alata* (Mignouna et al. 2002c). The saturation of these maps with polymorphic markers, such as simple sequence repeat (SSR) is necessary for full utilization of their potentials. The identification of key traits that are related to yield and quality of yams, and the gene action associated with the traits are paramount for the understanding of the genetics of this important crop. Previous studies have focused mainly on the analysis of disease resistance, and the identification of genomic regions associated with resistance to yam mosaic virus and anthracnose diseases in *D. rotundata* and *D. alata*, respectively (Mignouna et al. 2002a, b, c, d). Results of these analyses, however, are yet to be confirmed in populations of different genetic background and also in other environments. Presently, IITA is involved in the identification of key traits that affect yield and quality of yam, with the hope of identifying DNA markers that are linked to these traits for their subsequent use in yam breeding. Various mapping populations are being developed for quantitative trait loci (QTL) analysis of yield and quality-related traits in *D. rotundata* and *D. alata*.

4.3 Crop Improvement Through Traditional and Advanced Tools

There has been very limited use of wild *Dioscorea* spp. in yam improvement program, although farmers, mainly in West Africa, practice domestication of wild species to develop cultivated forms (Dumont et al. 2006). In addition, wild-related species possess useful traits. *D. abyssinica* and *D. praehensilis*, the wild relative of guinea yams (*D. rotundata–D. cayenensis* species complex) are believed to be reservoir of resistance genes for virus diseases such as Yam mosaic virus; however, their use in breeding programs has been almost negligible. Similarly, the related species of Asiatic yam (*D. alata*) such as *D. nummularia* and *D. transversa*, may possess resistant genes for anthracnose disease, but has been seldom used in breeding programs. It is believed that there may be introduction of deleterious characteristics into the cultivated species if crossing is made using wild species. Additionally, there may be problems of interspecific hybridization owing to differences in ploidy level between different species, and this may require embryo rescue technique for the success of such crosses. In general, few efforts have been made to use wild species in breeding programs.

Traditional breeding efforts in yam have resulted in substantial achievement leading to release of high-yielding and diseases-resistant cultivars. For instance, through classical breeding, IITA has developed several clones and populations of *D. rotundata* and *D. alata*, and disseminated these for further evaluation and selection under local environmental conditions in partnership with national programs in Africa. Through collaborative evaluation of IITA-derived breeding lines with National Research Institutes (National Root Crop research Institute, Umudike, Nigeria, and the Crops Research Institute of Ghana), eight varieties of *D. rotundata*, seven during 2001 and 2003 and one in 2007, have been released in Nigeria and Ghana, respectively, while three varieties of *D. alata* were released in Nigeria in 2009. More lines are in the pipeline to be released by these institutions and other root crop programs in other yam-producing countries including Benin, Burkina Faso, Ivory Coast, Sierra Leone, Togo, and Liberia. These varieties have multiple pest and disease resistance, wide adaptability, and good organoleptic attributes (Mignouna et al. 2007).

Yam-breeding programs have focused on clonal selection from landraces and hybridization of elite genotypes within and between species. IITA yam research focuses mainly on the most important species cultivated in Africa, *D. rotundata* and *D. alata*. There are many wild yam species available, some of them with edible tubers, which may be potential sources of useful traits, which can be used in breeding programs.

4.3.1 Interspecific Hybridization

Interspecific hybridization in yams is desirable for the genetic improvement of the crop, but it is faced with a lot of challenges, including cross-compatibility and synchronization of flowering, and very little work has been done in these areas. Some species can hybridize easily, while others do not. For instance, *D. rotundata* can be crossed to *D. cayenensis*, but crossing either of the two to *D. alata* has not been successful

(IITA unpublished data). Research effort in interspecific hybridization at IITA is geared toward the genetic improvement of yam, primarily on *D. rotundata*, *D. cayenensis*, and *D. alata* by transferring complementary traits from one to the other, e.g., higher carotenoid in *D. cayenensis* could be transferred to *D. rotundata* by interspecific hybridization.

4.3.2 Advanced Biotechnological Techniques for Yam Improvement

Research on biotechnology of yam has been limited to tissue culture, but has focused on disciplines including genetic transformation and molecular marker application. Xinhua et al. (1986) and Schafer et al. (1987) attempted to transform *D. opposita* and *D. bulbifera* using *Agrobacterium* followed by Tor et al. (1993, 1998) who transformed cultures of *D. alata* using particle bombardment. However, genetically modified yam is yet to be developed although this approach could be successfully used in transferring virus resistance and anthracnose resistance genes into commercial varieties. Similarly, some progress has been made using isozymes (Hamon and Touré 1990a, b; Dansi et al. 2000a, b) and DNA markers (Terauchi et al. 1992; Ramser et al. 1996, 1997; Mignouna et al. 1998; Chaïr et al. 2005) for germplasm characterization and phylogenetic studies in yam. Mignouna et al. (2007) demonstrated the relationship between cultivated yams (*D. cayenensis* – *D. rotundata*) and wild species from West Africa using DNA markers.

4.3.2.1 Callus Culture and Plant Regeneration

Direct plant regeneration from explant materials, somatic embryogenesis, and plant regeneration from callus culture have been very useful for rapid clonal propagation and obtaining somaclonal variants. In yam, regeneration rate is very low in some cases, and there are genotype-dependent differences between species in their ability to generate plantlets in vitro (Asiedu et al. 1998). These techniques have been conducted on several species: *D. rotundata* (Ng 1984; Osifo 1988), *D. alata* (Mantell et al. 1978; Fautret et al. 1985; Twyford and Mantell 1990), *D. composita* and *D. cayenensis* (Sinha and Chaturvedi 1979; Viana and Mantell 1989), *D. abyssinica* (Ng et al. 1994) and *D. trifida* (Fautret et al. 1985).

4.3.2.2 Embryo Culture

Embryo rescue, a technique to save immature embryos from a hybrid and to enhance germination of seeds that are dormant or cannot germinate easily under normal situation, has been reported in Chinese yam (Yakuwa et al. 1981) and *D. rotundata* (Okezie et al. 1983).

4.3.2.3 Protoplast Culture

Protoplast isolation and culture technique is very useful in the production of yam varieties, especially those that do not produce flowers. These techniques offer the greatest promise for the production of somatic hybrids, but very little work has been reported in this research area. They have been successfully applied to *D. rotundata* (Onyia et al. 1984) and *D. alata* (Twyford and Mantell 1990).

4.3.2.4 Development of Expressed Sequence Tags

The lack of DNA or express sequence tag (EST) sequences hampered fundamental studies such as gene characterization and genetic linkage mapping in yams. The dearth of genomic data in yam species prompted IITA to take up initiative in generating fundamental molecular genomic data useful to enhance the conventional yam improvement program. Most of the currently available molecular markers for the yam genomes are based on amplified fragment length polymorphism (AFLP) and random amplified polymorphic DNA (RAPD). For marker-assisted breeding to be feasible, it is important to develop user-friendly and high-throughput markers such as EST-SSR and single nucleotide polymorphism (SNP) markers. The application of genomic tools in yam breeding and germplasm characterization will accelerate the development of improved yam germplasm in Africa and elsewhere.

In an effort to develop the yam genomics resources, an initial attempt was made to sequence ESTs from a

cDNA library constructed from floral tissue (Mignouna et al. 2003). However, the first several hundred sequences were found to be predominantly house-keeping genes suggesting a better approach in construction of cDNA library. Another project has been launched recently to generate several thousand ESTs in a collaborative project between IITA and the University of Virginia (based on USAID-Linkage fund). The objective of the project was to generate cDNA libraries from yam leaf tissues challenged with *Colletotrichum gloeosporioides*, the fungal pathogen responsible for yam anthracnose disease, and perform the sequencing of cDNA clones to subsequently identify ESTs with differential gene expression for marker development. In addition, SSR markers were developed in *D. alata* using sequence resources from heterologous crop species. Some of these markers were polymorphic in the test panel and are presently being tested in IITA's Central Biosciences Center.

4.4 Linkage Mapping and QTL Analysis

Two framework linkage maps were constructed for wild yam species, *D. tokoro*, using 271 AFLPs, five sequence-tagged microsatellite sites, one isozyme, and one morphological marker. For the two parents DT7 and DT5 used in the cross, 13 and 12 linkage groups, respectively, were identified. The total map lengths were 669 and 613 cM, respectively, for DT7 and DT5, which covered more than 75% of the *D. tokoro* genome (Terauchi and Kahl 1999). Similarly, maps were constructed in *D. alata* using AFLP markers that included 338 markers on 20 linkage groups with a total map length of 1,055 cM (Mignouna et al. 2002b) and in *D. rotundata* in which 107 markers were mapped on 12 linkage groups (585 cM) for male and 13 linkage groups (700 cM) for female (Mignouna et al. 2002c). They identified three quantitative trait loci (QTLs) and one QTL for resistance to Yam mosaic virus on male and female parent linkage groups, respectively. Similarly, one AFLP marker was found to be associated with anthracnose resistance on linkage group 2 explaining about 10% of total phenotypic variance (Mignouna et al. 2002a). Another linkage map was generated for *D. alata* based on 508 AFLP markers that covered a total length of 1,233 cM on 20 linkage groups accounting for about 65% of the entire genome of yam (Mignouna et al. 2007). Similarly, Petro et al. (2009) developed a linkage map of *D. alata* using 523 polymorphic markers from 26 AFLP primer combinations that covered a total length of 1,627 cM and included 20 linkage groups. They detected 10 QTLs for anthracnose resistance explaining 7–40% of the phenotypic variance. One of the major objectives of future research on linkage mapping should be integration of all the available maps of *D. alata* and *D. rotundata* with that of wild diploid species, *D. tokoro*. Although AFLP markers have been used for generating linkage maps so far, efforts are underway to saturate the maps with SSRs or EST-SSRs for use in yam breeding programs.

Bulked segregant analysis (BSA) has been successfully used in *D. rotundata* and *D. alata* to identify Yam mosaic virus (YMV) and anthracnose resistance genes, respectively (Mignouna et al. 2002a, b). Two RAPD markers OPW18850 and OPX15850 closely linked in coupling phase with the dominant YMV-resistance locus *Ymv-1* were identified. Similarly, two RAPD markers, OPI171700 and OPE6950, closely linked in coupling phase with anthracnose resistant gene, *Dcg-1*, were identified.

4.5 Application of Genomic Tools and Gene Discovery

Few laboratories around the world work on the molecular aspects of yam species in general and uncultivated yams, in particular. With respect to genomic resources, yam is considered as one of the understudied crops. Yam scientists who intend to embark on marker-aided breeding will begin the step with searching for nucleotide sequence data on the web. In this genomic era when genome sequences of many plants are completed or being completed every now and then and web-accessible databases are growing exponentially, it is hard to find new entries of nucleotide or protein sequences of any of the yam species. A number of peer-reviewed public web accessible databases offer the tool to seek information on specific genomic resources and tools (Galperin 2008). Entrez (http://www.ncbi.nih.nil.gov) is one of the popular web resources that comprise a wide array of primary and secondary databases and tools for data mining. According to the latest record of Entrez, draft genomes have been completed or are near completion for a

number of crop plants and model plants. Unfortunately, yam is not one of the 137 species listed in the Entrez plant genome project database. However, the Germplasm Resources Information Network (GRIN) database (USDA-ARS 2009) comprises 126 species of *Dioscorea*, the largest of the five genera in the family Dioscoreaceae. A summary of the data records for the family Dioscoreaceae in the GRIN database is shown in Fig. 4.4. These figures match the Genbank records but slight variation exists in the number of nucleotides and protein accessions in the genus *Dioscorea*, which is 576 and 588, respectively, in Genbank. Most of the genomic data in the family Dioscoreaceae come from *Dioscorea elephantipes* (a wild species), whose entire plastid genome has been sequenced (Hansen et al. 2007). Hence, most of these accessions represent house-keeping genes or photosynthesis-related genes of non-nuclear genome origin.

Nucleotide sequence search in the most recent release of genbank database (GenBank release 169.0) showed a total of 771 nucleotide sequences and 634 protein sequences for the entire family of Dioscoreaceae. Of these, while only 31 are mRNA (EST) sequences, the remaining genomic sequences are predominantly partial sequences of house-keeping genes derived from organelle genomes – chloroplast and mitochondria (Table 4.2). All of the 31 EST sequences (acc DN792550–DN792580) were obtained from subtracted cDNA library of *D. nipponica* root. In the absence of nuclear genome data, the plastid genome sequences can be utilized for applications such as DNA bar coding for species identification (details below).

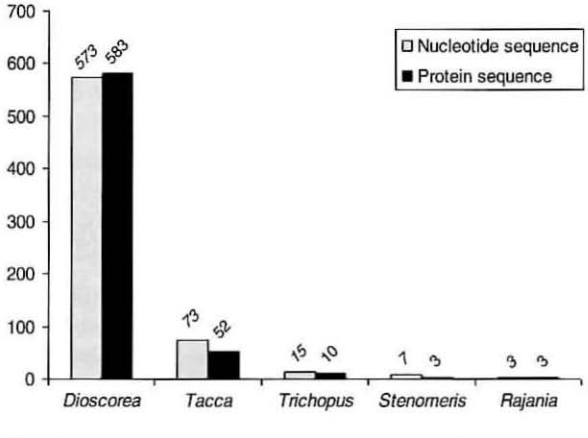

Fig. 4.4 Nucleotide and protein sequences in different genera within the family Dioscoraceae

To date, less than 20 microsatellite markers are available in yam species. Tostain et al. (2006) have isolated a total of 16 microsatellites containing sequences from *D. alata* (5 SSRs), *D. abyssinica* (6 SSRs), and *D. praehensilis* (5 SSRs). These SSR markers were tested and found to be transferable to other *Dioscorea* species. The remaining cultivated species such as *D. rotundata* and *D. cayenensis* have significantly low number of sequence data (3 and 6, respectively). Furthermore, most of the Genbank accessions in these two species were non-nuclear genomes. In general, a large number of the available genomic data in *Dioscorea* were obtained from non-cultivated species (Table 4.2). This information can still be utilized in cultivated species by applying homology-based techniques such as cluster of orthologous groups (COG) for primer design and other applications (Tatusov et al. 1997, 2003).

4.5.1 Genome Size

Plant DNA C-values database (Bennett and Leitch 2005; http://data.kew.org/cvalues/) contains DNA C-values for 11 yam species, all from the genus *Dioscorea*. It is notable that the Mbp values span a wide range of values from as low as 466 (1C = 0.48 pg) in *D. togoensis* to 2,352 (1C = 2.4 pg) in *D. villosa* (a wild *Dioscorea* species). The difference in genome size is partly attributable to the ploidy level even though values are not available for some species to conclude with certainty (Table 4.3). Despite the apparent variation in the estimated values, in general, the genome size of yam species is relatively low.

4.5.2 Plastid Genome

The *D. elephantipes* annotated chloroplast complete genome was reported by Hansen et al. (2007) (Genbank: EF380353; RefSeq: NC_009601). The entire plastid size is 152,609 nucleotides consisting of 129 genes of which 83 are protein-coding genes. These entries represent the most annotated records of the

Table 4.2 The number of nucleotide and protein entries in Genbank for the genus *Dioscorea* as of August 2010

Dioscorea sps.	No of nucleotide entries	Species	No of protein entries
Wild			
D. tokoro	87	*D. tokoro*	40
D. elephantipes	12	*D. elephantipes*	241
D. polystachya	73	*D. polystachya*	7
D. communis	22	*D. communis*	10
D. nipponica	18	*D. nipponica*	8
D. schimperiana	21	*D. schimperiana*	12
D. zingiberensis	13	*D. zingiberensis*	4
D. chouardii	10	*D. caucasica*	5
D. abyssinica	9	*D. gracillima*	4
D. sansibarensis	8	*D. sansibarensis*	4
D. praehensilis	8	*D. tenuipes*	6
D. sylvatica	8	*D. sylvatica*	4
D. pyrenaica	8	*D. decipiens*	4
Cultivated			
D. bulbifera	50	*D. bulbifera*	45
D. japonica	34	*D. japonica*	11
D. alata	87	*D. alata*	7
D. trifida	11	*D. sp. Qiu 94044*	4
D. rotundata	3	*D. rotundata*	0
All other taxa (including 31 EST sequences)	351	All other taxa	207
Total	771	Total	634

Table 4.3 Estimated genome sizes of *Dioscorea* species listed in Plant DNA C-values Database release 4.0, October 2005 (Bennett and Leitch 2005; http://www.kew.org/cvalues/homepage.html)

Genus	Species	Chromosome No.	Ploidy level	1C (Mbp)	1C (pg)	Original Reference
Wild						
Dioscorea	*togoensis*	40[a]	4	466	0.48	Hamon et al. (1992)
Dioscorea	*abyssinica*	40[a]	4	613	0.63	Hamon et al. (1992)
Dioscorea	*mangenotiana*	40[a]	4	613	0.63	Hamon et al. (1992)
Dioscorea	*praehensilis*	40[a]	4	613	0.63	Hamon et al. (1992)
Dioscorea	*sylvatica*	NA[b]	NA	833	0.85	Bharathan et al. (1994)
Dioscorea	*villosa*	NA[b]	NA	2352	2.4	Bharathan et al. (1994)
Dioscorea	*elephantipes*	NA[b]	NA	6615	6.75	Zonneveld et al. (2005)
Cultivated						
Dioscorea	*alata*	40	4	564	0.58	Arumuganathan and Earle (1991a, b)
Dioscorea	*cayenensis-rotundata*	40	4	613	0.63	Hamon et al. (1992)
Dioscorea	*cayenensis-rotundata*	60	6	858	0.88	Hamon et al. (1992)
Dioscorea	*cayenensis-rotundata*	80	8	1274	1.3	Hamon et al. (1992)

[a]Numbers refer to references listed at http://data.kew.org/cvalues/updates.html#REFERENCES of the C-values database (Bennett and Leitch 2005)
[b]Data not available

genus *Dioscorea* in public database. Information from the plastid genome has been successfully utilized for phylogenetic studies such as estimation of relationships among the major angiosperms, and provided an insight into the evolution of gene and intron content (Jansen et al. 2007; Hansen et al. 2007). Additional sequences and annotation of the genus *Dioscorea* can be found on the GOBASE database (http://gobase.bcm.umontreal.ca/). A search for *Dioscorea* in this database returns 615 chloroplast genes.

4.6 Future Perspective of Genomic Research

The paucity of genomic resources in yam species calls for a concerted effort from the research community to enrich genomic resources of yam so as to accelerate the germplasm enhancement endeavor. The rate of bolstering yam genomic resources is steadily growing at IITA. EST-derived SSR markers are already in use to characterize germplasm. Furthermore, this EST project is anticipated to result in the development of microarrays for high-throughput expression analysis and gene discovery. However, the outcome of this project is still too little to impact the deployment of molecular markers in advanced molecular-assisted breeding. The importance of yam in sub-Saharan Africa warrants the application of relevant cutting-edge technologies for germplasm enhancement. The steadily declining cost of the new generation sequencing technologies provides an impetus to embark on yam genome-sequencing initiative. Presently, a staggering number of plants are undergoing genome sequencing. As one of world's most important food crop, it is imperative to consider genome sequencing of yam. Successful completion of genome sequencing and annotation will trigger other investigators involved in evolution, taxonomy, physiology, systems biology, and comparative genomics. In parallel with development of genomic resources, thorough cytogenetic studies are of paramount importance to understand the genome structure and to pave the way for successful and effective subsequent genomic studies such as gene/QTL mapping and genome sequencing. Some of the ongoing initiatives in molecular technology are discussed below.

4.6.1 Microarray

Generation of sufficient nucleotide sequences paves the way for global gene expression analysis via microarray. The current EST project in yam is anticipated to generate sufficient ESTs to build microarray chips for transcript analysis. However, a concerted effort to generate DNA, mRNA, and protein data is the best way for accelerated development of genomic tools in yam species.

4.6.2 Identification of Candidate Genes by Comparative Genomics

In order to overcome the paucity of gene level knowledge in yam, approaches such as resistance gene analogs (RGAs) can be deployed to identify genes involved in plant defense (Moroldo et al. 2008). The rapid accumulation of genome sequence data sparked an array of functional genomics tools that are being employed to understand the complex pathways involved in host plant–pathogen interaction. In the absence of yam genome sequences, such homology-based identification of RGAs can be utilized as a shortcut method for the identification of gene-targeted markers of economically important diseases such as yam mosaic virus and yam anthracnose.

Application of comparative genomics will further allow the transfer of knowledge from thoroughly studied model plants to yam. Discovery of genes involved in flowering in model plants such as *Arabidopsis* have been successfully utilized to identify homologous genes in garlic (Rotem et al. 2007) and in cauliflower (Saddic et al. 2006). Such approaches can be adopted for discovery of genes regulating the flowering signaling pathways in yam. Dormancy, described as the temporary suspension of growth in stored yam tubers, is a persistent challenge that could be tackled by genomic intervention.

4.6.3 Reverse Genetics: Tilling

Targeting induced local lesions in genomes (Tilling) is increasingly becoming a popular technique of reverse genetics for detection of mutation in a target gene followed by assignment of the phenotypes to the gene sequence (Gilchrist and Haughn 2005). Tilling has been applied to crops with insufficient DNA sequences information by comparative genomics. Application of Tilling seems very prudent for yam researchers by capitalizing on advances in functional genomics of model plants. Knowledge of gene function in highly investigated plants sheds light on the genetic mechanism and pathways of key physiological traits in under-researched crops such as yam. Some of the traits that can be targeted by tilling could be flowering, dormancy, and resistance to diseases caused by fungi and viruses such as *Yam mosaic virus*.

4.6.4 DNA Barcoding

Species identification in the genus *Dioscorea*, the most important and the largest in the family, has remained a daunting task and the consequences of domestication on species identification has been described above. In IITA, there is a growing interest to apply DNA barcoding not only to address the issues with mislabeling and understanding interspecific crosses, but also to get an insight into the ongoing domestication process in *Dioscorea*. A DNA barcode is an aid for taxonomic identification that uses short, standardized DNA sequences (mostly 400–800 bp) present universally in target lineages and has sufficient sequence variation to discriminate among species of a particular organism. This provides a rapid and accurate procedure for unambiguous species identification by having sufficient sequence variation among species and low intraspecific variation. The universally accepted genes for plant DNA barcoding are of plastid origin. Polymorphism of chloroplast DNA especially *trn*K, *mat*K, and intergenic *trn*L–*trn*F regions have been used to study the phylogeny of various plants (Wolfe et al. 1987; Kress et al. 2005; Selvaraj et al. 2008). Fortunately, yam has relatively well-developed chloroplast genome information that can be tapped (Hansen et al. 2007).

4.7 Recommendations and Way Forward

Advances in cytogenetics such as molecular cytogenetics including techniques such as comparative genomic hybridization arrays (CGH), SNP-array based karyotyping, and automated systems for counting the results of standard FISH preparations, promise easy, accurate, and fast cytogenetics studies. These tools must be employed as a matter of urgency to re-examine the cytogenetics of yams. SSR markers, chromosome counts, and flow cytometry have been used with success to determine the mode of inheritance and the level of ploidy and provide new evidence for a base chromosome number (Bousalem et al. 2006). This approach must be adopted to analyze all yam species especially the wild types to clarify once for all the cytogenetic status to pave way for accelerated improvement of the yam crop.

Efforts for ex situ conservation of yam need to be augmented to protect the diversity of landraces and wild yams which are under threat of extinction due to agriculture intensification, erosion of forests, and increase in incidence of pests and diseases. There is a need for understanding the diversity in pathogen population responsible for important diseases such as "anthracnose" and "mosaic" in yam in order to establish an efficient breeding tactics. This information will also help in characterization of wild species for resistance to pests and diseases. Promising germplasm that has broad-specific resistance identified during this process could augment pest- and disease-resistance breeding programs.

Numerous questions meanwhile remain to be answered as far as the cytogenetics of yams is concerned. For instance, as Scarcelli et al. (2005) queried, "could the coexistence within the genus *Dioscorea* of the diploid $2n = 40$ *D. rotundata*, with diploid $2n = 20$ *D. tokoro* and *D. gracillima* be due to diploidization of genomes after their polyploidization for wild and cultivated plant genomes as documented for soybean?" What is the inheritance pattern for polyploidy in yams? Could diploidy and monoecy of parental plants be related? What is the chromosome number? A wide range of biotechnological and bioinformatics tools could be adopted to address these recalcitrant issues that will unravel the genetic potential of this orphan crop, which offers food and income security to millions of subsistence farmers in tropical and subtropical world.

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