Chapter 23
Genomics of Yams, a Common Source of Food and Medicine in the Tropics

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Abstract Yams (*Dioscorea* spp., Dioscoreaceae), grown either for their starchy tubers or medicinal properties, are important crops in the tropics and subtropics. Yams broaden the food base and provide food security and income to over 300 million people. They are vegetatively propagated and comprise both diploid and polyploid species. Despite their economic and socio-cultural importance, very little is known about the genetics and genomics of yams due to research neglect and several biological constraints. Consequently, conventional breeding efforts have been severely hampered. Research to unravel the apparent complexity of the yam genome will have far-reaching implications for genetic improvement of this important tuber crop. Nevertheless, progress has been made recently towards understanding *Dioscorea* phylogeny and phylogenetic relationships within the genus. Also, improved molecular technologies have been developed for genome analysis, including germplasm characterization, cytogenetics, genetic mapping and tagging, and functional genomics. Genetic linkage maps have been constructed for *D. rotundata* and *D. alata*, and quantitative trait loci associated with resistance to *Yam mosaic virus* in *D. rotundata* and anthracnose (*Colletotrichum gloeosporioides*) in *D. alata* have been identified. In addition, candidate random amplified polymorphic DNA markers associated with major genes controlling resistance to *Yam mosaic virus* and anthracnose have been identified. These markers could be converted to sequence-characterized amplified regions and used for marker-assisted selection for resistance to diseases. An initial cDNA library has been constructed to develop expressed sequence tags for gene discovery and as a source of additional molecular markers. Genetic engineering offers a powerful tool, complementing conventional breeding approaches, for yam improvement. Methods for yam transformation, including in vitro plant regeneration, gene delivery, selection of transformed tissues, and recovery of transgenic plants have been developed but still need improvements. This chapter reviews advances made in yam molecular marker development for genome

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analysis, phylogeny, molecular cytogenetics, characterization of genetic diversity, genetic mapping and tagging, and progress in functional genomics.

23.1 Introduction

Yams are classified in the genus *Dioscorea*, a genus widely reported as comprising around 600 species (Burkill 1960). More recent estimates indicate that approximately 200 species are distributed throughout the tropics and subtropics (Ayensu 1972). Plants of the genus *Dioscorea* are angiosperms that belong to the monocotyledon order Dioscoreales. Interestingly, the order Dioscoreales is characterized by several dicotyledonous features, such as reticulate-veining, stalked net-nerving leaves, circularly arranged vascular bundles in the stem, and the lateral position of the pistil. Yams show a second vestigial cotyledon, which renders them intermediate with respect to the phylogenetic relationships between mono- and dicotyledonous plants, even though the traditional division of the angiosperms in mono- and dicotyledonous plants was formally discontinued with the introduction of the Magnoliopsida as a distal class of the angiosperms (Frohne and Jensen 1998). Yam plants are herbaceous or woody climbing plants with tuberous, starch-rich storage organs. The aerial storage organ of Dioscoreaceae is the bulbil. They are perennial plants with a strongly marked annual cycle of growth (Coursey 1983). In the southern United States the name yam is used for sweet potato (*Ipomoea batatas*, L. Poir.) and elsewhere for the edible tubers of aroids (Frohne and Jensen 1998; Purseglove 1988). More generally, and in the present chapter, the term yam is confined to plants of the genus *Dioscorea*. Guinea yams (*D. rotundata* and *D. cayenensis*) were domesticated in West Africa, while the water or greater yam (*D. alata*) probably originated from the southeast Asian-Oceanian region (Malapa et al. 2005). *D. alata* was previously considered to be a possible cultigen (Barrau 1965), but it is now known to be a true species with normal sexuality (Lebot et al. 1998; Malapa et al. 2005).

In West and Central Africa, where Guinea yams were domesticated about 7000 years ago, farmers selected genotypes that best suited their needs and thus have generated a large number of traditional cultivars. Yam production has increased steadily in the last decade, from 18 million metric tonnes in 1990 to recent estimates of over 39 million (FAO 2006). This increase has been achieved mainly through the planting of traditional landraces and can be explained by the rapid increase in acreage of yam fields into marginal lands and into non-traditional yam growing areas. This expansion highlights the need to provide farmers with improved varieties that combine high yields with pest and disease resistance and acceptable tuber quality.

Collaborative evaluations of International Institute of Tropical Agriculture (IITA)-derived breeding lines with national yam programs in Africa have led to the official release of a number of white yam varieties having multiple pest and disease resistance, wide adaptability, and good organoleptic attributes. However, this progress has been difficult, time-consuming, and laborious due to biological constraints that impede the elucidation of the genetics of important traits in yam. Genetic

improvement of yam has been hampered by a long growth cycle (lasting about eight months or more), dioecy, poor to no flowering, asynchronous flowering of male and female parents, polyploidy, vegetative propagation, high heterozygosity, and poor knowledge of the crop's genetic diversity (Asiedu et al. 1998). Yam is cultivated in widely varying agroecological zones and the performance of genotypes is disparate across regions, thereby multiplying breeding goals.

Molecular markers that are linked to genes controlling economic traits would be useful in selection at an early stage of the plant's growth, thereby enhancing the speed and efficiency of selection. Biotechnology not only provides an alternative approach, but also complements the efforts in conventional breeding (Mignouna et al. 2003a). This chapter will review yam molecular marker development for genome analysis, phylogeny, cytogenetics, characterization of genetic diversity, genetic mapping and tagging, and progress in functional genomics.

23.1.1 Economic, Agronomic, and Societal Importance of Yams

Yam is produced throughout the tropical and sub-tropical regions of the world. Guinea yams are the most popular and economically important yams in West and Central Africa where they are indigenous, while water or greater yam is the most widely distributed species globally. The majority of global yam production is in Africa. West Africa accounts for about 95% of world production and 96% of the area (FAO 2006). Yam production globally reached 39.85 million Mt harvested from 4.44 million ha in 2005 (FAO 2006). The largest producer was Nigeria with 26.59 million Mt, followed by Ghana (3.89), Côte d'Ivoire (3.00), and Benin (2.56). The profitability of yam production, the value of yams in local trade (Hahn et al. 1987; Nweke et al. 1991), as well as the current and potential revenue from their export to ethnic markets in Europe and Northern America are often underestimated. In many parts of West Africa, for instance southeastern Nigeria, yams rank first among the major food crops in terms of cash income per hectare (IITA 1988; Nweke et al. 1991).

Food yams are grown principally for the carbohydrate they provide. The tubers, which are the only edible part, have a tremendous capacity to store food reserves. They broaden the food base and bring food security to 300 million people in the low-income, food-deficit countries of the tropics, providing them with about 200 kilocalories daily. The net dietary protein calorie content in yams is about 4.6%, which compares well with 4.7% in maize (Hahn et al. 1987; FAO 1999). Socioeconomic surveys conducted in Nigeria indicated that there was a positive elasticity of demand for yams at all expenditure levels, and that production research towards increasing yam supply will consequently increase quantities consumed at low-income levels in sub-Saharan Africa (Nweke et al. 1992).

In West Africa, yam tubers are typically boiled and pounded into dough for easy swallowing. In Madagascar, tubers of some species can be eaten raw (e.g., *D. soso*, *D. nako*, and *D. fandra*). Others are simply boiled or baked (e.g., *D. alata*),

while others need extensive preparation such as immersion in running water for 1–3 days or drying in the sun (e.g., *D. antaly*). *Dioscorea* species are not only known for their food value but also for their secondary metabolites. They contain steroidal saponins, diterpenoids, and alkaloids, which have been exploited for making poisons (Neuwinger 1996) and pharmaceutical products (Chu and Figueiredo-Ribeiro 1991).

23.1.2 Yam as an Experimental Organism

The genus *Dioscorea* has been considered to be an attractive model for investigating ploidy events and chromosome evolution in wild and cultivated species in relation to vegetative propagation and the process of domestication (Bousalem et al. 2006). Yam, though an "orphan" crop, can provide a good model for traits not possessed by other model crops. For instance, the tuber is an important ecological (and economic) trait possessed by only a few models: potato may serve for eudicots, but we have little basis to judge how suitable it might be as a model for monocots. In other words, we do not know how general the tuberization process is in angiosperms. Knowledge of gene expression at the appropriate stages in a tuberous monocot (e.g., *Dioscorea*, yams), matched with a candidate gene approach, would allow us to address this question. Phylogenetic morphology studies reveal that the "monocot" mode of leaf development typifies a nested group. However, not all monocots have this mode of leaf development; some have either dicot or intermediate modes of development. The grass models may serve taxa with monocot modes; but other taxa (e.g., *Dioscorea*) may be needed to understand other developmental modes (Bharathan 1996).

Given its dioecious nature with different morphologies of staminate and pistillate plants in some species; its dicot-like leaf structure (net-veined and petiolate) with early development intermediate between dicot and monocot modes (Bharathan 1996); distinct changes in shoot apical meristem (SAM) structure and phyllotaxy during phase transition from juvenile to adult (Burkill 1960); tuber formation and dormancy; small C-value and widespread polyploidy (Dansi et al. 2001; Egesi et al. 2002; Bousalem et al. 2006), *Dioscorea* offers a system in which to raise general biological questions that cannot be addressed in many other species. It thus holds great promise of yielding important clues to explain differences between eudicot and grass models (e.g., non-orthology of KNOX genes controlling SAM indeterminacy [Bharathan et al. 1999]) and offering examples of biological phenomena such as dioecy, tuberization, and modes of vine twining.

Tuber dormancy is an important field adaptive mechanism that also helps to maintain organoleptic quality during storage, but it creates a major problem for plant breeders. This is because harvested tubers remain dormant (i.e. incapable of developing an internal shoot bud or external shoot bud/sprout) for 30 to 150 d (Orkwor and Ekanayake 1998), only one crop cycle is possible per year, which slows progress in yam improvement. Knowledge gained from yams may lead to the elucidation and

successful manipulation of tuber dormancy in other plant species. Elucidation of the molecular changes taking place in yams during post-harvest storage will help in understanding the process of tuber dormancy (Kone-Coulibaly et al. 2003).

23.2 Development of Molecular Markers for Genome Analysis

Yams are monocots, but very distantly related to the grasses. Thus there is no convenient model system for yam genomics. Initial efforts in yam genomics sought to exploit heterologous DNA sequences as a source of RFLP markers (Terauchi et al. 1992). Later, the approach of using uncharacterized DNA sequences was adopted as a source of genetic markers. AFLP was the molecular marker of choice (Mignouna et al. 1998). RAPD and AFLP polymorphism was high among diverse yam species, with AFLP revealing the highest polymorphism. Sixty-four AFLP primer combinations were tested for their potential use in assessment of genetic diversity in white Guinea yam (Mignouna et al. 1998). Although RAPD markers were adequate for genetic diversity studies (Dansi et al. 2000a), the level of polymorphism detected in mapping populations was low; therefore, RAPD was not considered a good marker-system for mapping purposes. Contrary to RAPDs, the high level of polymorphism revealed by AFLP markers, coupled with their robustness, made AFLP a more reliable and reproducible marker-system for yam genome analysis (Mignouna et al. 1998; Mignouna et al. 2003b; Malapa et al. 2005).

As progress was being made in yam genomics, co-dominant molecular markers such as microsatellites or simple sequence repeats (SSRs) were required because of their expected high polymorphism, co-dominant inheritance, high abundance and even distribution across the genome. In a study of a natural population of *D. tokoro*, a wild diploid East Asian yam species ($2n = 20$), Terauchi and Konuma (1994) detected microsatellite polymorphisms. A high number of polymorphic alleles was detected per microsatellite locus, suggesting that these microsatellite primers could be transferable to other *Dioscorea* species. Unfortunately, when the *D. tokoro* microsatellite primers were applied to other yam species, they failed to amplify any DNA, indicating that these primer sequences are not conserved among *Dioscorea* species. However, the study demonstrated the potential usefulness of these markers for yams. Microsatellite markers were later developed for food yams in a collaborative project between IITA and the University of Saskatchewan, Canada, and used to assess genetic diversity in *D. rotundata* (Mignouna et al. 2003b). A few microsatellite markers were characterized by several authors, but because of the relatively small number of markers developed (six in *D. tokoro* [Terauchi and Konuma 1994] and nine in *D. rotundata* [Mignouna et al. 2003b]) and the low level of polymorphism detected in mapping populations, microsatellites were not considered a good marker system for mapping purposes.

Increased interest in yam genomics and the need for robust molecular and genetic tools for genome analysis led to the development of 10 microsatellite markers in *D. japonica* (Mizuki et al. 2005). Tostain et al. (2006) developed and characterized

16 new SSR markers in different species of yam, several of which were transferable to species of other *Dioscorea* sections. Transferability was higher among species belonging to the same botanical section (*Enantiophyllum*. Within the Enantiophyllum section, the patterns differed for the African species on one hand and the Asian-Oceanian species *D. alata* and *D. nummularia* on the other. Similarly, Hochu et al. (2006) developed 20 microsatellite markers in American yam (*D. trifida*) and found high cross-species amplification involving four additional *Dioscorea* species: the cultivated *D. alata, D. cayenensis–D. rotundata,* and the two African wild yams, *D. praehensilis* and *D. abyssinica*. The four species tested are classified into the botanical section *Enantiophyllum* that is phylogenetically distant from the section *Macrogynodium* to which *D. trifida* belongs. This large cross-species applicability indicated that the primers will be useful for additional studies within the *Dioscorea* genus.

23.3 Phylogeny, Molecular Cytogenetics, and Genetic Diversity

23.3.1 Yam Phylogeny

Phylogenetic relationships of yams have not been well established because of difficulties in species identification due to a high level of polymorphism in morphological characters. Although all species in the genus are dioecious, some species have different species names for its male and female plants. Recent analyses of morphological and molecular data sets have indicated relationships within Dioscoreaceae R. Br. (Caddick et al. 2002a), and a formal reclassification of the family has been presented (Caddick et al. 2002b). Dioscoreaceae now contains four distinct genera, *Dioscorea, Stenomeris, Tacca* (previously in Taccaceae), and *Trichopus*. The dioecious Dioscoreaceae genera, *Borderea, Epipetrum, Nanarepenta, Rajania, Tamus*, and *Testudinaria*, are nested within *Dioscorea* in phylogenetic analyses (Caddick et al. 2002a), and are therefore sunk into it.

Wilkin et al. (2005) conducted phylogenetic analysis of yams based on sequence data from the plastid genes *rbcL* and *matK*, using 67 species of *Dioscorea* and covering all the main Old World and selected New World lineages. They found that the main Old World groups (such as the right-twining *Dioscorea* section *Enantiophyllum* to which most edible yams belong) are monophyletic and that there are two distinct lineages among the endemic Malagasy taxa. These findings have important consequences for character evolution, intrageneric classification, and the origins of diversity in *Dioscorea*. Earlier, Kawabe et al. (1997) had examined the phylogenetic relationship of six species (*D. gracillima*, *D. nipponica*, *D. quinqueloba*, *D. septemloba*, *D. tenuipes*, and *D. tokoro*), in the section *Stenophora* of the genus *Dioscorea*, based on nucleotide sequence variation in 1073 bp of the coding region of the phosphoglucose isomerase locus. They found that *D. tenuipes* and *D. tokoro* belonged to a monophyletic clade, while the other species formed a separate monophyletic group. These studies point to the possibility of greatly simplifying the classification of yams proposed by Knuth and Burkill (Chaïr et al. 2005).

Based on RFLP analysis of the chloroplast and nuclear ribosomal DNA, Terauchi et al. (1992) found four different taxonomic groups with*D. rotundata* and *D. cayenensis* being classified in the same chloroplast DNA-defined group as the wild species *D. praehensilis, D. abyssinica,* and*D. liebrechtsiana*. The other three classes identified among the wild species comprised *D.minutiflora, D.burkilliana, D.smilacifolia,* and *D.togoensis*. Cluster analysis based on the enzyme system 6-PGD revealed a tendency towards separation of the annual species (*D. abyssinica*, *D. praehensilis*, *D. rotundata*) from the perennial species (*D. burkilliana*, *D. smilacifolia*, *D. minutiflora*) and their derivative (*D. cayenensis*) (Mignouna et al. 2003c). This indicated that 6-PGD may be useful in phylogenetic studies in yam.

23.3.2 Molecular Dissection of the D. cayenensis-rotundataComplex

Ayensu and Coursey (1972), Martin and Rhodes (1978), and Miège (1982a, b) proposed merging of Guinea yams, *D. cayenensis* and *D. rotundata*, into a species complex based on a comparison of their morphological characteristics. However, the taxonomy and evolution of the *D. cayenensis-rotundata* complex remains controversial (Dansi et al. 1999), with different authors considering Guinea yam to be represented either by one species, two species, or a species complex (Martin and Rhodes 1978; Miège 1982a, b; Onyilagha and Lowe 1985; Hamon and Touré 1990a, b; Hamon et al. 1992; Terauchi et al. 1992; Asemota et al. 1996). Cluster analysis of 467 Guinea yam accessions based on seven polymorphic enzyme systems clearly separated the *D. rotundata* (white yam) and the *D. cayenensis* (yellow yam) accessions (Dansi et al. 2000b). This clear partition into two groups was consistent with the concept that the two forms of Guinea yam represent different genetic entities which may be treated as two separate taxa, supporting the view of Onyilagha and Lowe (1985).

Molecular markers have been used to delineate species boundaries surrounding *D. rotundata* and *D. cayenensis* (Terauchi et al. 1992; Mignouna et al. 1998; Mignouna et al. 2005a, b; Chaïr et al. 2005). On the basis of RFLP analysis of chloroplast and nuclear ribosomal DNA, Terauchi et al. (1992) proposed that *D. rotundata* was domesticated from one of the wild species that shared the same chloroplast genotype, and that *D. cayenensis* is of hybrid origin and should be considered as a variety of *D. rotundata*. Similar results were obtained by Chaïr et al. (2005), who reported that *D. cayenensis* and *D. rotundata* share the same cpSSR haplotype. However, Ramser et al. (1997) used four molecular marker systems (RAPD, microsatellite-primed PCR random amplified microsatellite polymorphism, and a comparative sequence analysis of three noncoding chloroplast DNA sequences) to confirm the separation of Guinea yams into two distinct species, *D. rotundata* and *D. cayenensis*. Mignouna et al. (1998) used two AFLP primer combinations to generate a total of 87 polymorphic loci across 20 Guinea yam cultivar groups. Phylogenetic analysis of the data revealed five major cultivar groups among which the group that corresponded to *D. cayenensis* was genetically

distant from the varietal groups of *D. rotundata*, as found in other molecular studies. In another study with RAPD and double stringency PCR markers (Mignouna et al. 2005a), accessions of Guinea yam, which were classified into seven morphotypes/cultivar groups, could be clearly separated into two major groups corresponding to *D. rotundata* and *D. cayenensis*. It was proposed, based on these results, that cultivars classified into *D. cayenensis* should be considered as a taxon separate from *D. rotundata.* Mignouna et al. (2005a) considered that the discrepancy between their results and those of Terauchi et al. (1992) probably arose from the fact that they scanned the entire genome using PCR-based markers while the RFLP analysis of Terauchi et al. (1992) was based on the rDNA gene. Although useful for inferring phylogenetic relationships, the rDNA gene represents only a small fraction of the total genome and there are risks of recreating gene trees rather than species trees.

23.3.3 Molecular Cytogenetics

Identification of the most common gametic ploidy level of each accession in a polyploid species, such as yams, is necessary for efficient hybridization. It is of practical importance for yam breeders to determine the ploidy status of clones, especially of new introductions, before they can be utilized in a breeding program, to enable matching of ploidy levels as well as facilitate ploidy manipulations in intraspecific crosses. The existence of various ploidy levels and the lack of a diploid relative to the cultivated polyploid yams have greatly complicated genetic studies of the crop. Unlike most plants, differences in ploidy levels in yam plants are not reflected by any characteristic morphological feature. Phenotypic differences are expectedly greater within than between ploidy levels as also observed in other species (Dessauw 1988). Thus, cytological irregularities leading to erratic flowering and reproductive behavior are expected. 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 (Abraham and Nair 1990; Abraham 1998) cells. However, because yam chromosomes are small, generally dot-like, and most often clumped, determining ploidy levels by counting chromosomes is tedious and difficult (Baquar 1980; Zoundjihekpon et al. 1990).

Our current knowledge of yam ploidy is based on the basic chromosome number of 10 or nine, with a high frequency of polyploid species (Essad 1984; Zoundjihekpon et al. 1990; Gamiette et al. 1999; Dansi et al. 2000c, 2001; Egesi et al. 2002). Tetraploid species are the most frequent, followed by 6x and 8x forms in similar proportions. The base chromosome number $x = 10$ is reported in all the Asian species, but is found in only 52% of the African species and 13% of the American species examined so far. The remaining African and American species are considered to have a basic number of $x = 9$ (Essad 1984). In segregating populations of water yam (*D. alata*) and white Guinea yam (*D. rotundata*) ($2n = 4x = 40$), the observed segregation of AFLP markers reflected a disomic inheritance

(Mignouna et al. 2002a, b). These results indicated an allotetraploid structure for *D. rotundata* and *D. alata*. However, segregation analysis using isozyme and microsatellites markers led to the conclusion that *D. rotundata,* belonging to the botanical section *Enantiophyllum*, is a diploid species (Scarcelli et al. 2005). *D. trifida* was considered to be an octoploid species with 80 chromosomes (x = 10) (Esad 1984). In microsatellite segregation analysis, individual patterns showed a maximum of four alleles, strongly suggesting that *D. trifida* is a tetraploid species with 2n = 4x = 80 chromosomes (Hochu et al. 2006). Bousalem et al. (2006) used cytogenetic evidence to show that the species is autotetraploid with a basic chromosome number of x = 20. Interestingly, Segarra-Moragues et al. (2004) concluded that the two species of the Bordera section, *D. pyrenaica* and *D. chouardii* (Caddick et al. 2002b) endemic to the Pyrenees (Spain and France), are allotetraploid with the chromosome base number of x = 6, which was not previously reported within the Dioscoreaceae. The finding of two new basic chromosome numbers, x = 6 (Segarra-Moragues and Catalán 2003; Segarra-Moragues et al. 2004) and x = 20 (Scarcelli et al. 2005), raises questions on the validity of the current ploidy data in the genus *Dioscorea*. If these new basic chromosome numbers are confirmed in a larger number of yam species, that should lead us to reconsider the basic chromosome number of yams on a more general level and, as a consequence, to decrease the level of ploidy of at least some species.

23.3.4 Genetic Diversity

Molecular markers are increasingly being used to examine the genetic diversity of cultivated and wild yam species (Mignouna et al. 2005b). Dansi et al. (1999) used a comparative morphological study to establish linkages between Guinea yam morphotypes/cultivar groups and their wild relatives. RAPD markers showed considerable variability when used for cultivar identification of Jamaican yam cultivars belonging to five food yam species: *D. alata*, *D. cayenensis*, *D. esculenta*, *D. rotundata*, and *D. trifida* (Asemota et al. 1996). The usefulness of RAPD as a discriminative and informative marker system in yam was also demonstrated by Ramser et al. (1996) using 23 *D. bulbifera* accessions collected from different geographic locations in Africa, Asia, and Oceania. That study also provided evidence in support of an earlier proposal of the independent domestication of this species in Africa and Asia.

Mignouna et al. (1998) found one varietal group among germplasm originating from Cameroon clustered separately from all other West African genotypes, indicating that this group constitutes a separate gene pool, which could be useful for genetic improvement of West African *Dioscorea* germplasm. A study to investigate the genetic relationships among West and Central African *D. rotundata* germplasm revealed a low level of genetic similarity between the yam accessions, with each genotype being identified as a unique individual using the three marker assays (Mignouna et al. 2003b). This study confirmed the high intraspecific variation within *D. rotundata* reported by Asemota et al. (1996), Mignouna et al. (1998),

and Dansi et al. (2000a, b). Tostain et al. (2006) surveyed the diversity at 10 microsatellite loci for 146 *D. rotundata* accessions from Benin and the diversity of six microsatellite loci on 56 others. A significant excess of heterozygotes was observed at nine of the 15 polymorphic loci, which is expected in this vegetatively propagated crop. The significant excess of homozygotes, estimated at two loci, could be explained by the presence of null alleles.

Malapa et al. (2005) showed that *D. alata* is a heterogeneous species that shares a common genetic background with *D. nummularia*. Cluster analysis, using UPGMA (unweighted pair group method with arithmetic mean) based on AFLP profiles, revealed the existence of three major groups of genotypes within *D. alata*, each assembling accessions from distant geographical origins and different ploidy levels. Lebot et al. (1998) found no correlations between morphotypes, chemotypes, and zymotypes of 269 cultivars of *D. alata* (originating from the South Pacific, Asia, Africa, and the Caribbean), which were analyzed with four enzyme systems, including 6-PGD. The existing genetic variation is believed to be due to sexual recombination imposed by outcrossing (Lebot et al. 1998; Malapa et al. 2005).

Mignouna et al. (2005a) investigated genetic relationships among wild and cultivated yams in Nigeria and found that *D. rotundata* cultivars appeared most closely related to *D. praehensilis* and *D. liebrechtsiana* De Wild. *D. abyssinica* was widespread in the northern savannahs of the country. Similar to the situation with *D. praehensilis*, cultivars classified in 10 cultivar groups were morphologically very similar to *D. abyssinica* and might have been domesticated from this species (Chaïr et al. 2005). Isozyme analysis of wild yam species from Côte d'Ivoire revealed three groups: annual, semi-perennial, and perennial. Some cultivated accessions clustered with annual wild species, whereas others clustered with semi-perennial or perennial species (Hamon 1987). For Miège (1968), *D. burkilliana* and *D. minutiflora* are two morphologically very close species that differ only by the characteristics of their below-ground parts. However, Mignouna et al. (2003c) used 6-PGD isozyme analysis to show that the two species are genetically distinct. The principal species associations revealed by cluster analysis were *D. abyssinica*/*D. praehensilis*, *D. liebrechtsiana*/*D. praehensilis*, *D. mangenotiana*/*D. praehensilis*, *D. rotundata*/*D. praehensilis, D. cayenensis*/*D. burkilliana*.

There is unanimity among farmers and considerable agreement in research findings (Hamon 1987; Terauchi et al. 1992) that all the cultivated forms of the *D. cayenensis*/*D. rotundata* complex are the products of an ancient, or more or less recent, domestication of the four major wild species *D. abyssinica* Hochst, *D. praehensilis* Benth., *D. burkilliana* Miège, and *D. mangenotiana* Miège) a process that is still in progress in certain parts of West and Central Africa (Dumont and Vernier 2000; Mignouna and Dansi 2003; Scarcelli et al. 2006a, b). Mignouna and Dansi (2003) called for a revision of the taxonomy of *Dioscorea* species because they found it difficult to understand how individuals identified in the wild as *D. praehensilis* or *D. abyssinica* can directly become *D. rotundata* or *D. cayenensis* following "domestication" without any genetic change. In fact, Mignouna and Dansi (2003) showed that predomesticated yam plants could not always be clearly identified as belonging to either wild or cultivated species.

To assess the effect of farmers' practices on the diversity of *D. cayenensis–D. rotundata* cultivars, Scarcelli et al. (2006a) used AFLP analysis of a total of 213 yam accessions consisting of predomesticated yams, *D. cayenensis–D. rotundata*, *D. abyssinica,* and *D. praehensilis*. Of the 32 predomesticated accessions, 16% clustered with *D. praehensilis*, 37% with *D. abyssinica,* and the remaining 47% with *D. cayenensis–D. rotundata*. They thus demonstrated the use of wild plants by farmers in their domestication process and showed that through domestication farmers influence and increase the genetic diversity in yam by using sexual reproduction of wild and possibly cultivated yams. In a related study on the impact of ennoblement of spontaneous yams on the genetic diversity of yam in Benin, Scarcelli et al. (2006b) used 11 microsatellite markers to analyze yam tubers from a small village in northern Benin and demonstrated that wild × cultivated hybrids are spontaneously formed. Many of the spontaneous yams collected by farmers from surrounding savannah areas for ennoblement were shown to be wild and hybrid genotypes. They demonstrated that some yam varieties have a wild or hybrid signature and performed a broader-ranging genetic analysis on yam material from throughout Benin, which revealed that ennoblement is practiced in different ecological and ethno-linguistic regions. By maintaining a mixed yam propagation system (sexual cycle and asexual propagation), farmers ensure widespread cultivation of the best genotypes while preserving the potential for future adaptation. The mechanism underlying phenotypic modifications during "domestication" is unknown. They could result from phenotypic plasticity, epigenetic modifications, or somatic mutations. The latter two explanations are compatible with the fact that morphological changes are maintained through vegetative multiplication.

23.4 Genetic Mapping and Tagging in Yam

Molecular genetic maps and marker-aided analysis of complex traits can be used to elucidate the genetic control of yield potential and tuber quality and to locate genes of pest and disease resistance, nutrient use efficiency, tuberization, and flowering. For these reasons, a concerted effort to map the yam genome and dissect the inheritance of complex traits was initiated at IITA. It was anticipated that cultivated yams would have their origin from a cross between genetically distinct individuals, so the alleles derived from each parent may be different. One general approach to mapping plants of this type is to examine the genotypes of selfed progeny; however, this is not feasible for dioecious yams, so the approach taken was to generate multiple F_1 individuals derived from crosses between the same parents, male or female. F_1 mapping populations of *D. alata* and *D. rotundata* were subjected to in vitro micropropagation based on techniques developed by Ng (1992). *D. rotundata* populations segregated components of resistance to *Yam mosaic virus* (YMV), genus *Potyvirus* (Mignouna et al. 2001b), while the *D. alata* populations segregated for yam anthracnose disease resistance (Mignouna et al. 2001a).

YMV is a major limiting factor for stable production of yams and *D. rotundata* is particularly susceptible to the virus (Thouvenel and Dumont 1990). A study of the genetic control of YMV resistance in three *D. rotundata* cultivars to a Nigerian isolate of YMV showed that resistance is manifested differentially as the action of a single dominant gene in simplex condition or a major recessive gene in duplex condition (Mignouna et al. 2001b). The dominant locus that contributes to YMV resistance was tentatively named *Ymv-1* until tests of allelism are conducted. Anthracnose disease, caused by *C. gloeosporioides* (Abang et al. 2003), is a major constraint to the production of yam worldwide (Winch et al. 1984; McDonald et al. 1998), with *D. alata*, the most widely distributed species, being particularly susceptible to the disease. Initial genetic inheritance studies showed that resistance to yam anthracnose in *D. alata* is dominantly but quantitatively inherited (Mignouna et al. 2001a). A single major dominant locus controlling resistance in the breeding line TDa 95/00328 was tentatively designated *Dcg-1* until allelism is investigated. The efficiency and effectiveness of breeding for YMV and anthracnose resistance will be greatly improved by marker-assisted selection based on genetic mapping of major genes controlling the resistance.

23.4.1 Linkage Mapping

Chromosome pairing in tetraploids can occur such that only homologues pair or such that any two homeologues may pair. These two types of pairing have very different consequences for segregation patterns so that these plants may, in the extreme, exhibit either diploid or tetraploid genetics. Intermediate types of behavior may also occur. Thus it was important to establish which type of segregation was being observed in the cultivated yams. Genes controlling important traits such as yield, tuber quality, and pest and disease resistance are usually distributed among several quantitative trait loci (QTLs), which may not be linked, thus making these traits difficult to manipulate using conventional breeding methods. The recessive nature of YMV resistance in some *D. rotundata* genotypes means that such resistance cannot be easily tracked at the phenotypic level, demanding refined diagnostic procedures such as molecular mapping for detailed genetic localization of specific genes (Mignouna et al. 2001b). Screening by molecular markers linked to QTLs has the advantage of selecting pairs of parents with genes at different loci for the same trait (Solomon-Blackburn and Barker 2001).

Genetic mapping using AFLP led to construction of the first, separate, comprehensive, molecular linkage maps of *D. rotundata* and *D. alata* (Mignouna et al. 2002c, d). The *D. rotundata* map was based on 341 co-dominantly scored AFLP markers segregating in an intraspecific F_1 cross (Mignouna et al. 2002d). Separate maternal and paternal linkage maps were constructed, comprising 12 and 13 linkage groups, respectively. The mapping population was produced by crossing a landrace, TDr 93-1, as female parent and a breeder's line, TDr 87/00211, as the male parent. The markers segregated like a diploid cross-pollinator population, suggesting that the

D. rotundata genome is an allotetraploid (2n = 4x = 40). More recent findings have confirmed that *D. rotundata* is a diploid species (Scarcelli et al. 2005). Three QTLs with effect on resistance to YMV were identified on the maternal linkage map, while one QTL for YMV was detected on the paternal linkage map (Mignouna et al. 2002d). These results showed that both parents contributed to resistance in the progeny.

Similarly, a genetic linkage map of the water yam (*D. alata*) genome was constructed based on 469 co-dominantly scored AFLP markers segregating in an intraspecific F_1 cross (Mignouna et al. 2002c). The F_1 was obtained by crossing two improved breeding lines, TDa 95/00328 as female parent and TDa 87/01091 as the male parent. The 469 markers were mapped on 20 linkage groups with a total map length of 1,233 cM. Again, the markers segregated as in a diploid cross-pollinator population, suggesting that the water yam genome is allotetraploid (2n=4x=40). One QTL located on linkage group 2 was found to be associated with anthracnose resistance, explaining 10% of the total phenotypic variance (Mignouna et al. 2002c).

Conservative estimates put the genome coverage of the *D. rotundata* and *D. alata* maps at 56% and 65%, respectively. There are several reasons why the maps may not give complete coverage. The most obvious is that the two parents may have some common ancestry so that segments of the linkage maps may be devoid of polymorphism and thus cannot be identified in genetic analysis.

One approach towards gaining insights on this issue would be to align the *D. alata* and *D. rotundata* maps. This would give us additional confidence in the general map structures and enable the development of suitable markers for genomic surveys of other populations. An attempt was made to derive gene sequence-based markers, but unfortunately the cDNA library used for this analysis contained an unexpectedly high proportion of rRNA sequences. Nevertheless, this remains a viable objective, and would also permit the alignment of these maps with that recently presented for diploid *D. tokoro,* 2n=2x=20 (Terauchi and Kahl 1999). Both maps provide useful tools for further genetic analysis of agronomically important traits in yam. While AFLPs continue to be identified and used for mapping the yam genome, efforts are geared towards saturating the map with simple sequence repeats (SSRs) and expressed sequence tags (ESTs), for greater ease of application in yam breeding.

23.4.2 Gene Tagging

Bulked segregant analysis has been shown to be efficient for initial identification of disease resistance-linked markers. The approach has been successfully applied in yams for identification of YMV and anthracnose resistance genes (Mignouna et al. 2002a, b). Two RAPD markers. $OPW18_{850}$ and $OPX15_{850}$, closely linked in coupling phase with the dominant YMV-resistance locus *Ymv*-1 were identified. These markers successfully identified the resistance gene in resistant genotypes among a sample of 12 *D. rotundata* varieties (Mignouna et al. 2002b). Similarly, a single locus, *Dcg-1*, that contributes to anthracnose resistance was identified in

D. alata. Two RAPD markers, $OPI17_{1700}$ and $OPE6_{950}$, closely linked in coupling phase with *Dcg-1* were identified (Mignouna et al. 2002a). Both markers successfully identified *Dcg-1* in resistant *D. alata* genotypes among 34 breeding lines, indicating their potential use in marker-assisted selection (MAS). The RAPD markers identified in these studies will be made more reliable and specific and easier to apply for indirect selection by converting them into co-dominant PCR-based sequence-characterized amplified regions. Further AFLP mapping is planned to identify additional QTLs and strengthen existing marker-QTL linkages. Candidate gene analyses are yet to be employed to investigate a variety of traits. To date, significant associations have been demonstrated for disease resistance in numerous crops. The yam breeding program at IITA plans to use MAS for selecting parental lines for breeding purposes. It is likely that as QTL experiments are expanded, additional genes will be identified for use in breeding.

23.5 Functional Genomics

The development of genomic resources and technology is a major focus in the yam genetics and breeding community. A cDNA library, produced from male flowers, was constructed in Bluescript vector and used for EST analysis (H. Mignouna, unpublished data). This approach has proven to be efficient for gene identification, gene expression profiling, and cataloging. It also provides markers and resources for the development of cDNA microarrays. Microarrays are not yet available for yams, mainly because the number of available gene sequences is still very small. Two cDNA libraries, one each for *D. alata* genotypes resistant or susceptible to yam anthracnose disease, have also been constructed recently (based on total RNA isolated from young leaves) towards identification of clones that are differentially expressed in the two genotypes (Narina et al. 2007). The libraries from the resistant and susceptible genotypes now have 10,000 and 6,000 cDNA clones, respectively, which are being sequenced.

Another reliable and potentially powerful way to identify candidate loci controlling agronomic traits in yam is application of the cDNA/AFLP technique, which generates polymorphic transcript-derived fragments (TDFs) between the parents of a mapping cross. cDNA generated from total RNA was subjected to cDNA-AFLP techniques to gain molecular insights and identify differentially expressed genes up-regulated and down-regulated during the dormancy in yam tubers (Kone-Coulibaly et al. 2003). Two primer pairs were identified that had equal potential for producing the same number of TDFs in dormant yam samples. The resulting TDFs from postharvest-treated tubers will aid in the selection of putative up- and down-regulated fragments during yam dormancy. Once candidate genes have been identified, they can be employed in gene tagging and QTL mapping studies to look for associations between the candidate gene and the trait in question. The availability of a BAC library and the development of an effective system for transforming yam with large DNA fragments will provide conclusive evidence of the contribution of the candidate gene through complementation studies.

23.5.1 EST Development

The genome size of *D. rotundata* was estimated by Feulgen-stained root tip nuclei to be 0.8 pg per haploid nucleus, and thus is equivalent to the genome size of species such as rice, soybean, and spinach (Conlan et al. 1995). The current *D. rotundata* map covers a minimum of 56% of the yam genome. Based on the haploid nuclear DNA content of *D. rotundata* of 800 Mbp/1C, the physical distance per map unit could be estimated at 400 kb per cM, making map-based gene cloning feasible (Mignouna et al. 2002d). We have generated 1100 ESTs from cDNA clones randomly picked from libraries constructed from male flowers. However, most of the sequenced ESTs were either ribosomal or housekeeping genes. To understand the physiological complexity of the yam genome, expression and/or functional gene analyses need to be undertaken. Northern analysis and differential display PCR techniques could be used, but these techniques have limitations in the number of genes that can be analyzed simultaneously. There is a need to develop approaches such as the use of cDNA microarrays. Other plant microarrays could be evaluated for use. As pointed out earlier, the development of a large number of ESTs will allow larger scale expression analysis.

23.5.2 Transformation

Attempts have been made to develop *in vitro* breeding strategies (such as somatic hybridization and gene insertion techniques) to overcome breeding barriers and to hasten the genetic improvement of food yams. For instance, Mantell (1994) fused protoplast mixtures between disease-sensitive and disease-resistant clones of *D. alata* in attempts to develop somatic hybrids with increased tolerance to anthracnose. There is considerable scope for introducing specific genes encoding resistance to fungal diseases (i.e., glucanase, chitinase, and antimicrobial protein gene constructs) and to nonpersistently transmitted potyviruses (i.e., sense and antisense genes of the coat protein of yam mosaic viruses). Three prerequisites for applying genetic transformation for plant improvement are: (1) a reliable regeneration system that is compatible with transformation methods allowing regeneration of transgenic plants; (2) an efficient way to introduce DNA into the regenerable cells; and (3) a procedure to select and regenerate transformed plants at a satisfactory frequency (Birch 1997).

Early plant transformation experiments on yam were hampered by false positive transformants that were found to be due to endophytic bacteria which exist within aseptically micropropagated shoot cultures and which express β-glucuronidase (Tor et al. 1992). Eventually, Tor et al. (1993) successfully demonstrated stable genetic transformation of *D. alata* embryogenic cell suspensions using biolistic insertion methods. However, biolistic approaches have a number of disadvantages such as the production of chimeric colonies containing mixtures of transformed and non-transformed cells and the instability of such colonies to retain inserted genes once

antibiotic and/or herbicide selection conditions are withdrawn following plant regeneration. Later efforts gave rise to successful yam protoplast culture leading to cell regeneration and direct gene transfer into yam protoplasts (Tor et al. 1998). Embryogenic cell suspension protoplasts of *D. alata* cv. Oriental Lisbon were successfully transformed using a standard polyethylene glycol-mediated uptake method. The availability of a protoplast system for transient gene expression studies in yams is expected to speed efforts towards the transformation of these tuber crops. The functional expression of valuable disease resistance genes, such as viral coat protein genes of yam mosaic viruses in either sense or anti-sense configurations, and combinatorial chitinase, glucanase, and anti-microbial protein genes driven by a range of either dicot promoters (NOS and CaMV35S) or monocot promoters such as ubiquitin, actin, ricin, and *emu,* needs to be investigated.

A number of host defense genes that could be good candidates for use in yam transformation have been characterized. Five chitinase isoforms, designated A, E, F, H1, and G, from yam tuber have been purified and characterized (Arakane et al. 2000). Chitinases E, F, and H1 had the highest lytic activity against the pathogen *Fusarium oxysporum*, while chitinase E was shown to be a possible biocontrol agent against strawberry powdery mildew (*Spherotheca humuli*) (Karasuda et al. 2003). Yam chitinase E has a similar amino acid sequence to a reported family 19 chitinase from *D. japonica* (Araki et al. 1992). Mitsunaga et al. (2004) cloned and sequenced a class IV chitinase from yam (*D. opposita*). The deduced amino acid sequence showed 50 to 59% identity to class IV chitinases from other plants. The yam chitinase, however, had an additional sequence of eight amino acids (a C-terminal extension) following the cysteine that was reported as the last amino acid for other class IV chitinases; this extension is perhaps involved in subcellular localization. A homology model based on the structure of a class II chitinase from barley suggested that the class IV enzyme recognizes an even shorter segment of the substrate than class I or II enzymes. This might explain why class IV enzymes are better suited to attack against pathogen cell walls.

23.6 Perspectives

The development and application of biotechnology tools are necessary to complement field breeding of yams. Molecular approaches have the potential to make yam breeding more efficient to reduce the cost and time required to produce new varieties. However, understanding and exploiting the complexity of the yam genome for improved yield and quality of yams remains a huge challenge. Large-scale gene identification and mapping have taken place in a number of model plants (e.g., *Arabidopsis* and *Medicago*) as well as some important food crops (e.g., rice, soybean, tomato, and maize). Whole genome sequencing and expression analyses have been conducted in *Arabidopsis* and rice and offer opportunities to understand the biological complexity of other plant genomes. However, these advances are yet to benefit under-researched tropical food crops such as yams (Nelson et al. 2004).

Completed genome sequences provide templates for the design of genome analysis tools in "orphan" crops lacking sequence information. Feltus et al. (2006) have shown that conserved-intron scanning primers are an effective means to explore poorly characterized genomes. Genes involved in many biochemical pathways and processes are similar across the plant kingdom (Thorup et al. 2000). Functions such as gene regulation, general metabolism, nutrient acquisition, disease resistance, general defense, flowering time, and flower development are largely conserved across taxa. Comparative mapping studies reveal that gene order is conserved for chromosomal segments among grass species (Devos and Gale 2000), with weaker chromosomal colinearity between monocots and dicots (Bennetzen 2000). Given the unique position of yams between monocots and dicots, it is doubtful how the work on models such as *Arabidopsis* and *Medicago* will benefit the species (e.g., Conlan et al. 1995). Although *Dioscorea* is a complex and highly variable genus, with several aspects of its biology still unresolved, we consider that there is a case for the adoption of yam as a "model" for plant genomics.

Efforts in yam genetics and genomics should be pursued and we believe the following specific areas need to be addressed in the near future. There is still a paucity of information, and some of the reports are conflicting, on yam phylogeny and the evolution of *Dioscorea* based on morphological, cytological, and molecular data. In this regard, the importance of non heritable or heritable epimutations in the development of yams should be investigated. Also, there is need for comparative analysis of the genomes of potato (dicotyledon) and yam (monocotyledon). The relationship between monoecious plants of *D. rotundata* (Scarcelli et al. 2005) and their normally dioecious relatives deserves further examination, as well as the nature of spontaneous hybrids in sympatric populations of wild and cultivated yams in Africa (Scarcelli et al. 2006). Selection and domestication of other annual yam species, including several indigenous West African and Malagasy species, should be undertaken before the natural populations disappear. Intraspecific hybridization between genetically distant landraces should be continued; for instance, between early and late maturing varieties of *D. rotundata* or between *D. alata* with and without bulbils. Hybrids obtained from these crosses do not require embryo culture.

Genetic linkage mapping of the two most important yam species (*D. rotundata* and *D. alata*) should be pursued. Denser genetic maps of each species and a consensus map for both must be constructed for practical breeding and germplasm enhancement purposes. QTL mapping should be reactivated with the initial identification of markers linked to disease resistance genes. Candidate gene identification using microarray and other approaches should be conducted to pin down the genes or QTLs involved in important agronomic traits. BAC library construction should be initiated, and efforts towards establishing a system for yam transformation should now be given more impetus (Tör et al. 1998). Embryo rescue will enable yam breeders to successfully make wide crosses with a greater number of related species of wild yams and have access to a much wider range of genes that can be used for the genetic improvement of yams. Wide crosses and embryo culture hold great promise for the transfer of tolerance to biotic and abiotic stresses from wild relatives to cultivated yams. Research to better understand the biology and agronomy of

wild relatives will greatly facilitate efforts aimed at unlocking the genetic potential hidden in the wild yam germplasm.

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