Genomics for transforming yam breeding

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Breeding challenges in yam

Yam (*Dioscorea* spp.), a multi-species, polyploidy, and vegetatively propagated crop, is an economically important staple food for more than 300 million people in West Africa, Asia, Oceania, and the Caribbean. The five major yam-producing countries in West Africa (Bénin, Côte d'Ivoire, Ghana, Nigeria, and Togo) account for 93% of worldwide production. *Dioscorea rotundata* and *D. alata* are the species most commonly cultivated in West Africa[1]. The genetic improvement of yam is faced with several constraints, including the long growth cycle (about 8 months or more), dioecy, plants that flower poorly or not at all, polyploidy, vegetative propagation, heterozygous genetic background, and poor knowledge about the genetics of the crop[2]. Progress has been made in breeding to develop F1 full-sib mapping populations from crossing male and female parents of *D. rotundata* for traits

Strategizing genomics for precision breeding. Photo by L. Kumar.

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such as multiple tuber production, improved cooking quality, and virus disease resistance; and of *D. alata* for resistance to anthracnose, improved cooking quality, and reduced tuber oxidation[3]. These are valuable sources of populations for genetic analysis in yam for its improvement.

Current status of yam genomics

There is no convenient model system for yam genomics. In recent years, some progress has been made in the development of molecular markers to assess their potential for germplasm characterization and phylogenetic studies in *D. rotundata-cayenensis* and their wild progenitors, such as *D. abyssinica* and *D. prahensilis*. Two framework linkage maps were constructed using *D. alata* that included 338 AFLP markers on 20 linkage groups with a total map length of 1055 cM; and *D. rotundata* in which 107 AFLP markers were mapped on 12 linkage groups (585 cM) for the male and 13 linkage groups (700 cM) for the female. Three quantitative trait loci (QTLs) on the male and one QTL on the female were identified for resistance to yam mosaic virus (YMV). Similarly, one AFLP marker was found to be associated with anthracnose resistance on linkage group 2, explaining about 10% of the total phenotypic variance.

Another linkage map was generated for *D. alata* based on 508 AFLP markers that covered a total length of 1233 cM on 20 linkage groups, accounting for about 65% of the entire genome. Genes conferring resistance to YMV have been identified in *D. rotundata* and to anthracnose in *D. alata* by the successful use of bulked segregant analysis (BSA). 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 resistance gene, *Dcg-1*, were identified[2].

Enriching the repertoire of molecular markers

In an effort to develop additional genomics resources, IITA was involved in sequencing ESTs from a cDNA library constructed from floral tissue. However, the first several hundred sequences were predominantly housekeeping genes. Recently, in a collaborative project with University of Virginia through USAID-Linkage funds, several thousand ESTs were generated using cDNA libraries from yam leaf tissues challenged with *Colletotrichum gloeosporioides*, the fungal pathogen responsible for yam anthracnose disease. This resulted in the identification of >800,000 EST sequences, from which about 1152 EST-SSRs were generated in *D. alata* for use in a yam improvement program. Although AFLP markers have been used for generating linkage maps so far, efforts are under way to saturate the maps with these EST-SSRs to identify the genomic regions associated with resistance to anthracnose disease.

DNA barcoding

Species identification in the genus *Dioscorea* has remained a challenge when active domestication is continuing in several parts of West Africa. Research on DNA barcoding is under way using chloroplast markers (rbcL, matK, and trnH-psbA) to understand the phylogenetic relationship between different species and also

Designing molecular markers using a bioinformatics platform. Photo by A. Alonge.

to get an insight into the ongoing domestication process.

Whole genome sequencing

Important considerations for the whole genome sequencing of yam include the genome size, ploidy level, and availability of homozygous clones. Estimation of the genome sizes of various *Dioscorea* species showed widely variable figures: *D. alata* and *D. rotundata* have genome sizes of about 800 mega base pairs (Mbp). Recently, an initiative was launched at IITA in collaboration with the Japan International Research Center for Agricultural Sciences (JIRCAS) to complete the whole genome sequencing of *D. rotundata*. Preliminary data yielded reasonable sequences. Further work is in progress to generate additional sequence data from the BAC library to facilitate the assembly of the genome which will culminate in producing the first draft genome sequence of *Dioscorea* species. Additional genomic information produced by resequencing several breeding materials and a parallel project in transcriptome analysis are poised to result in the discovery of a large number of molecular markers and help in the annotation of the genome.

Transcriptome analysis

In contrast to the genome sequence, which is fixed and uniform in all cells of a particular organism, transcriptome refers to the study of the total set of transcripts (expressed genes) in a given cell/tissue at a particular developmental stage or external environmental condition that could influence the physiology of the cell/tissue. IITA, in collaboration with USDA-Agricultural Research Service, Stoneville, embarked on RNAseq, the latest revolutionary tool for

transcriptome profiling, based on differential gene expression for anthracnose disease. One of the expected outcomes of this project is to enrich the genomic resources available for yam improvement, including the discovery of SNPs. The latest informatics and statistical methods will be applied to saturate the available linkage map and high resolution mapping of the QTL(s) for anthracnose resistance in different genetic backgrounds.

Genotyping-by-sequencing

With advances in the next generation technologies, the costs of DNA sequencing have come down to such an extent that genotyping-by-sequencing (GBS) is now possible in almost all crops. IITA has recognized the potential of such innovative techniques in accelerating the breeding of clonally propagated crops, such as yam. Hence, in an ongoing USAID-Linkage project, a diverse panel of *D. alata* genotypes, including parents of available mapping population progenies segregating for anthracnose disease will be genotyped by sequencing to identify a large set of SNPs and determine the divergence among the parents.

Conclusions

To meet the steadily increasing demand, the viable approach is to adopt the innovative plant breeding strategies for yam that integrate the latest innovations in molecular technologies with conventional breeding practices. As efforts are under way to obtain the complete genome sequences and the development of additional genomic resources, the groundwork for deploying yam molecular breeding has been laid. With the availability of new genomic markers and GBS, it would be possible to fingerprint yam germplasm to identify duplicates/mislabeled accessions, to conduct diversity analysis and association mapping. As the genus *Dioscorea* contains several other useful species, comparative genomic tools can be used to transfer or deduce genetic and genomic information in other species.

References

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