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Plant Genetic Resources: Effective Utilization

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Abstract

Characterizing ~~and~~ better understanding the genome organization and differentiating identity of genotypes based on their morphology and genome characteristics are vital determinants in their commercialization, management of germplasm repositories, and genetic conservation. Morpho-agronomic characterization of plants is not always feasible or sometimes labor intensive. Employing chloroplast, mitochondrial, and nuclear genome diversity using molecular biology tools will enhance the effectiveness and efficiency of revealing identity differences between genotypes. Using organelle and nuclear genome diversity can also answer a broad range of genetic, evolutionary relationships, and ecological questions.

INTRODUCTION

Plant genetic resources are one of the most important aspects of agricultural society. The sustainability and productivity of ecological systems depend on their biodiversity. Hence, it is very crucial to investigate the biodiversity of plant genetic resources to meet food demand and food security. Plant genetic resources comprise landraces, modern and wild species, and their relatives. Due to (a) destruction of humid forests, (b) invasion by exotic plants, (c) urbanization, (d) changes in agricultural practices, and (e) long-term breeding efforts, loss of diversity in plant genetic resources is one of the biggest concerns today. For instance, genetic diversity currently used in cereals is much less than their wild relatives.[1] Consequently, a tremendous investment has been made in effective utilization of plant genetic resources research worldwide over the years.

The large-scale analysis of plant genome diversity with respect to plant morphology is one of the most important determinants for effective and efficient utilization of genetic resources. However, morpho-agronomic characterization and phenotypic profiling of most plants are labor intensive and time consuming. In addition, phenotypic profiling may not provide distinguishable and usable data. For instance, different ploidy levels in buffalograss [*Buchloe dactyloides* (Nutt.) Engelm.], a native North American C_4 turfgrass species with extremely favorable drought tolerance, cannot be distinguished morphologically.[2] Additionally, genetic information obtained from morphological traits is limited and environmental effects have a tremendous impact on expression of quantitative traits. Hence, increased information on degree and distribution of nuclear and organelle genome diversity is imperative to develop sampling strategies and base populations and to identify redundancies and genetic contamination in germplasm collections. The information on organelle and nuclear genome diversity are also important to fingerprint and quantify genetic drifts/shifts and manage gene pools, which are a source of alleles for sustainable genetic improvement of plant species.[2,3]

There are a number of molecular biology tools and analytical techniques to enhance the knowledge of nuclear and organelle genome diversity that help in defining the uniqueness of a species and their ranking and phylogenetic relatedness. Biochemical methods (based on protein and enzymes, isozyme or allozymes) and DNA-based techniques (novel global-scale technologies such as genome sequencing, gene expression analysis, and genetic engineering) have been applied in combination with traditional methods to evaluate biodiversity and differentiate the identity of genotypes.[4]

This entry discusses employing organelle [mitochondrial DNAs (*mt*DNA) and chloroplast DNA (*cp*DNA)] and nuclear genome diversity for effective and efficient use of plant genetic resources and molecular biology tools utilized for analyzing their diversity.

ORGANELLE GENOME DIVERSITY

Analysis of organelle and nuclear genomes and the information on the degree of diversity has been widely used at the interspecies and intergeneric level for investigating phylogenetic relationships, cultivar identification, the geographical distribution of the progenitor, domestication, and the evolution and natural history of plant species.[4,5] Due to their lower level of mutation rate, highly conserved organelle genomes are very well suited for fingerprinting

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genotypes and elucidating evolutionary relationships.[6] The distinct characteristics of plant mitochondrial and chloroplast genomes make it a powerful tool for population genetic analysis and tracing maternal lineages in most angiosperms. Organelle genome analysis is also very important to compare coding and non-coding regions, which may provide more information regarding rearrangements and mutations at the species level.[7]

Unlike the nuclear genome that is bi-parentally inherited, the chloroplast genome is uniparentally, mostly maternally, inherited.[8] This characteristic of the chloroplast genome is very important to identify specific parentage of hybrid species and thus can be traced to matriarchal lineages. The chloroplast is highly abundant in leaves and therefore isolation of large quantities in pure form is relatively easy,[9] and its sequence appears to be highly conserved in terms of size, structure, gene content, and order. The chloroplast genome size, ranging from 120 to 218 kb in higher plants,[10] is small enough to resolve the fragments but is large enough to get taxonomic information.[11] One of the most attractive features of chloroplast genome is that it is independent of polyploidy that is very important in the evolution of plants. However, allo- and autopolyploidy needs to be identified for accurate analysis because alloploidy involves hybridization of diverged taxa[10,11] that affects the inheritance of chloroplast genome. The majority of studies using sequence data from *cp*DNA have been focused on phylogenetic work at fairly high taxonomic levels (intergeneric and above). However, recently, primer pairs for *cp*DNA and sequencing of organelle genomes and the detailed analysis of homologies between plastid and nuclear genomes are of interest to elucidate evolutionary history of plant species.[7] For instance, the plastid *matK* gene has a high rate of substitution when compared to the other chloroplast genes and evolves about three times faster than the widely used *rbcL* and *atpB* genes.[7,12] Hence, the *matK* gene has been extensively used in plant species to elucidate plant evolution and address phylogenetic questions at various taxonomic levels.

The mitochondrial genome has not been used extensively to study genome diversity. There is less knowledge available on mitochondrial genomes, and these genomes are not considered to be as conserved as the chloroplast genome. Most plant mitochondrial genomes are too large to allow for entire genomes to be characterized.[11] In addition, the mitochondrial genome is less abundant in leaves, which makes nucleic acid extraction more difficult. Yet, the study of *mt*DNA sequences in the nucleus to identify and trace specific genes is a powerful tool for identifying new source of alleles and creating genetic diversity for genetic improvement of plant species. Mitochondrial DNA sequences can easily be used for identification of genetic shifts/drifts, for a better understanding of organelle genome structure and their differences for gene tracing, and for differentiating the identity of genetic materials.[7,13]

NUCLEAR GENOME DIVERSITY

Higher mutation rates of plant nuclear genomes[6] make nuclear genome diversity very helpful for revealing the identity of genotypes and ex situ conservation of plant genetic resources. Genetic fingerprints developed through nuclear genome diversity are used to establish the origin of the specific plants and the relatedness of one genotype to another. A rational classification, which is necessary for many collection of plant species that exist around the world, is possible by employing nuclear genome diversity. Isolation of large quantities of nucleic acids from the nuclear genome is easy. However, study of single copy genes is challenging because nuclear genomes are larger than organelle genomes and the presence of organelle DNA sequences in the nuclear genome is also sometimes a big problem. A wide range survey of angiosperms indicated a high frequency of functional, single-gene transfers from mitochondrial to nuclear genomes during evolution.[14] By analyzing nuclear genome diversity, geneticists and plant breeders can predict expected properties of the progeny by tracing the presence or absence of certain forms of genes. Knowledge of nuclear genome diversity will also help researchers to predict important phenotypic properties, such as tolerance to biotic and abiotic stresses.

OTHER USES OF NUCLEAR AND ORGANELLE GENOME DIVERSITY

Nuclear and organelle genome variation can be used to identify the geographical and ecological distribution of various plant species and their relatives. Certain plant genetic resources from one region can be separated by genetically distinct resources from other regions. This is very important to acquire and harbor germplasm with useful and rare genes for widening the genetic base, maintaining diversity, and understanding the dynamics and biological function of biodiversity in natural and agricultural ecosystems. Investigation of organelle and nuclear genome diversity can also provide taxonomic relationship, which will be necessary for conservation strategies. This information assists in developing in situ (maintaining genetic resources in native habitat where they occur) and ex situ (maintaining genetic resources outside the native habitat) conservation strategies for effective and efficient utilization of plant genetic resources. A suitable conservation and management strategy will help promote an effective and efficient use of plant genetic resources without wasting resources through the high cost of management, and identification of genotypes with enhanced agronomic traits, including maturity (flowering), grain yield, disease resistance, and stress resistance. Manipulation of these traits would certainly have tremendous impact on efficient use of plant genetic resources. Taxonomic relationships provide information on identification of genomic homologies among the plant species to

devise an appropriate breeding approach and a better understanding of gene transfer from one species to another.

In molecular systematic studies, the discrepancy between the phylogeny based on nuclear, mitochondrial and chloroplast DNA data sets has been reported.[7,15] The most extensive differences are seen in comparisons between chloroplast and nuclear genome data and have been reported by a number of studies conducted using different plant species. The reason for this discrepancy is due to the likelihood that the nuclear genome and chloroplast genome had different evolutionary histories. It may also depend on introgressions or due to sorting of ancestral lineages.[15] This discrepancy is most serious at higher ploidy levels. Hence, solving these problems and having a better understanding is very important to accurately resolve problems of family and species relationships and taxonomic relatedness.

Analysis of nuclear and organelle genome diversity within and among plant species provides a suitable sampling strategy for germplasm acquisition. For instance, if most of the nuclear or organelle diversity is within populations when compared among populations, the sampling and conservation emphasis should be focused on collecting larger numbers of plants from few populations. On the other hand, emphasis should be placed on collecting a small number of plants from a large number of populations if any genome diversity is higher between populations.

~~MOLECULAR BIOLOGY~~ TOOLS USED FOR ORAGANELLE AND NUCLEAR GENOME DIVERSITY

Molecular biology tools have not only introduced new characters for the analysis of genome diversity, but they have provided characters that are not influenced by environment as is the case with morphological traits. PCR-based markers such as amplified fragment length polymorphism (AFLP), inter simple sequence repeat (ISSR), simple sequence repeat (SSR), sequence-related amplified polymorphism (SRAP), sequence tagged microsatellites (STMS), minisatellites, random amplified polymorphic DNA (RAPD), and single nucleotide polymorphism (SNP) have been extensively used to gain a better and enhanced knowledge on the nuclear genome variation and phenetic relationships among a broad range of plant species and subpopulations of single species[4] at the genomic level (Figs. 1 and 2).[2] PCR amplification of buffalograss genomic DNA from nine genotypes using two SRAP primer combinations is depicted in Fig. 2. Expressed sequence tags (ESTs) and putative genes controlling agronomic traits in genome sequence databases have been continually produced and are important resources to aid in exploiting wild relatives of plant species, which is an additional source of genes for domesticated plants.[16] The disadvantage of EST-SSR is that it is limited to the species where the sequence database is present. Transferability of molecular markers such as EST-SSR markers is very helpful for comparisons of genome structure among related species, high-resolution comparative maps and estimate genetic variation, which is indispensable to identify and manage unique plant genetic resources.

Universal primers that are available for amplification of specific organelle genome are being widely used in PCR-based RFLP analysis for either total DNA or extracted organelle DNA. Primers will work either directly, or with small alterations, across broad taxa. Organelle genome diversity can be assayed by direct sequencing for identification of new alleles and tracing genes during evolution. With genome sequence in hand, scientists are provided knowledge of all the genes within a plant species that will allow for the identification of unique germplasm and the ability to trace the ancestry of a specific gene in a gene pool.

Currently, the application of molecular biology tools has allowed scientists to remove species boundaries set by the traditional genetic improvement method of hybridization for a more effective utilization and improvement of plant genetic resources. Genetic engineering, embryo rescue,[17] somatic hybridization, and protoplast fusion techniques[18] are good examples of resolving problems of sexual incompatibility (begets the hybrid sterility) and lack of genetic recombination in distant wild relatives.[19] A gene from one organism can be transferred to any organism of choice for effective and efficient germplasm use. In vitro techniques through tissue culture has also proven to assist the conservation and management of plant genetic resources.

CONCLUSION AND PERSPECTIVES

Elucidation and an improved understanding of the degree and distributions of organelle and nuclear genome variation has enormous potential to benefit all phases of society, thus it provides improved, efficient, and effective genetics and breeding program. Understanding organelle and nuclear genome variation is ultimately needed to spawn a modern-day "Green Revolution" that is quite different from the Green Revolution of 40 years ago. The most exciting future prospects of employing a combination of both nuclear and organelle genome diversity consist of a comprehensive understanding of evolutionary relationship of plant genomes and their relationship with relatives. This will help in transferring new alleles from wild relatives to widely used plant species to effectively exploit wild germplasm resources. One should take care not to proclaim that organelle and nuclear genome diversity studies will feed the world, but that it will provide opportunities for improving plant genetic resources and will play a pivotal role in comparative studies in diverse fields such as ecology, molecular evolution, and comparative genetics. A challenge in the next decade will be to build integrated databases combining information on chloroplast, mitochondrial,

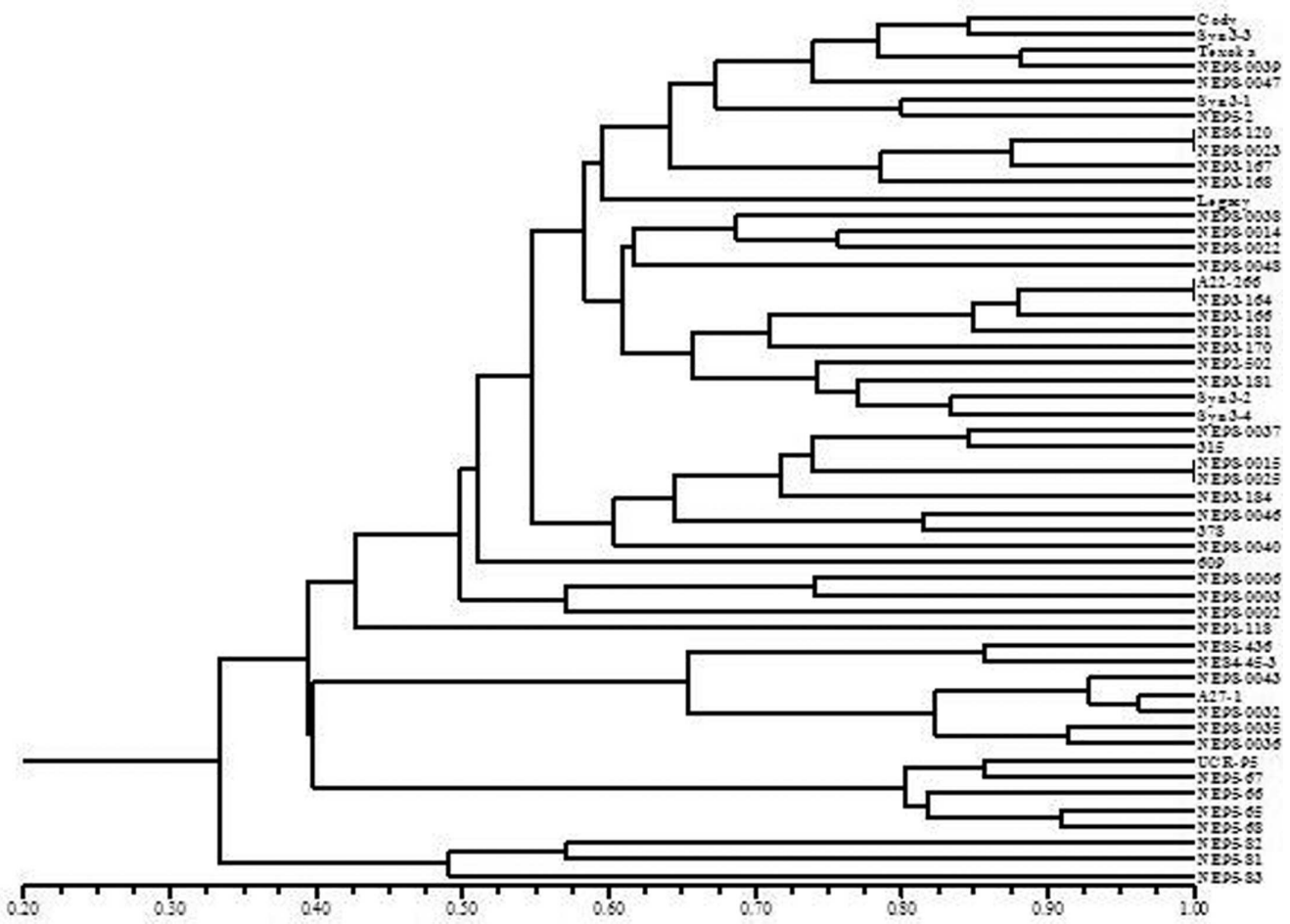

Fig. 1 An UPGMA dendrogram of genetic relationships among 53 buffalograss genotypes calculated based on genetic similarity by means of 34 SRAP primer combinations.[2]

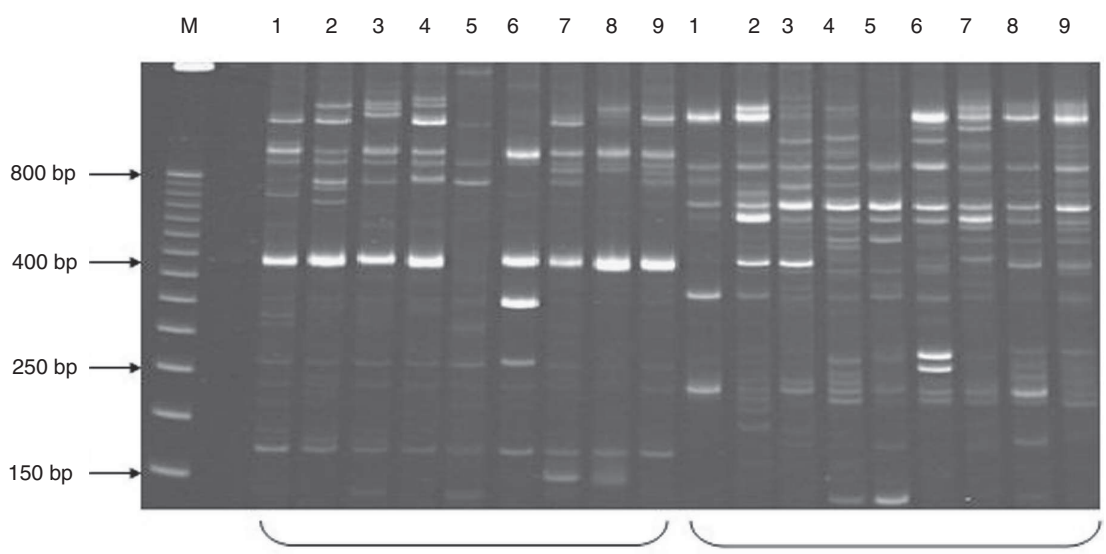

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Fig. 2 PCR amplification of buffalograss genomic DNA from nine genotypes. Lanes: 1 = Cody, 2 = Texoka, 3 = NE 98-032, 4 = NE 85-436, 5 = NE 93-184, 6 = NE 95-2, 7 = NE 98-043, 8 = NE 98-0015, 9 = Syn3-1, and lane M contains a 50-bp size marker (Promega Corp.). Two SRAP primer combinations, (a) Em7 + Me6 and (b) Em7 + Me5, were assayed. The DNA samples were fractionated in 12% non-denaturing acrylamide gels stained with ethidium bromide.[2]

nuclear, and phenotypic data for effective and efficient use of plant genetic materials.

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