International Journal of Agricultural Science Research Vol. 11(3), pp. 001-005, October, 2023
Available online at http://academeresearchjournals.org/journal/ijasr
ISSN 2327-3321 ©2023 Academe Research Journals

Review

Developments in Plant Genetic Studies through Intron Length Polymorphic Marker Analysis.

UNAMBA C.I.N

Department of Plant Science and Biotechnology (Botany), Faculty of Biological Sciences, Imo State University, Owerri, Nigeria.

Corresponding author. E-mail: chibuikem.unamba@gmail.com

Accepted 3 September, 2023

Advancements in plant genomics and genetics are crucial for breeding and cultivating crops resistant to pests, pathogens, and drought. Plant genetic analysis, based on genome sampling with informative genetic markers, is essential for studies of plant genetics, breeding, conservation, and evolution. Intron length polymorphic markers (ILPs) are useful markers for genetic diversity analysis, parentage analysis, gene mapping, and marker-assisted selection in plant genomes. They can be used for comprehensive studies on population genetics, gene flow, and evolutionary relationships within plant species. Integrating ILPs with other molecular markers can enhance the accuracy and efficiency of genetic mapping projects. Novel bioinformatics tools and standardized protocols for ILP marker development will be crucial for advancing plant genetic studies.

Keywords: Plant, Genetic, Intron Length Polymorphism, Markers, Diversity, Analysis

INTRODUCTION

Progresses in plant genomics and genetics, when applied in breeding, help support higher production and cultivation of crops resistant to pests, pathogens, and drought. Genome variation and diversity characterizations are important for the efficient and effective management of gene banks and for designing breeding programmes. Mondini *et al* (2009) posited that the evaluation of genetic diversity within and between populations may be measured using morphological, and biochemical characterization and evaluation and also characteristically done at the molecular level using various laboratory-based techniques such as allozyme or DNA analysis, which directly estimate levels of variation. Peterson *et al*., (2014) posited that plant genetic diversity analysis which depends on genome sampling with sufficient and informative genetic markers is an important component in studies of plant genetics, breeding, conservation and evolution. It is also imperative to have adequate diversity existing among cultivars or species to allow for the production of new varieties that are aimed toward crop productivity improvement. An insight into the molecular basis of the vital biological phenomena in plants is critical for the efficient conservation, management, and resourceful utilization of plant genetic resources.

Plant genetic studies have undergone significant advancements in recent years, particularly with the utilization of Intron length polymorphic markers (ILPs) to understand genetic diversity and evolutionary relationships within plant species. These studies primarily focus on exploring the variability present in non-coding regions of DNA, such as introns, which contain valuable genetic information that can be used for phylogenetic analyses and population genetics. By examining the variations in intron lengths among individuals or populations, researchers can gain insights into the genetic structure, gene flow patterns, and evolutionary history of plant species. These markers have proven to be valuable tools for understanding genetic diversity, population structure, and evolutionary relationships within plant species. By targeting intron regions, ILPs offer a higher level of polymorphism compared to traditional molecular markers, allowing for more precise genetic analyses. This has opened up new avenues for studying

plant genetics with a greater level of resolution and accuracy. Furthermore, ILPs are cost-effective, easy to use, and highly reproducible, making them an attractive option for researchers looking to explore the genetic makeup of plant populations.

Intron Length Polymorphic Markers: An Overview of Molecular Markers

DNA markers, also known as genetic markers or molecular markers, are short DNA sequences with a known location on chromosomes — a structure made of DNA and proteins. The marker is extensively used in labs as an identification tool for experiments like genetic mapping, DNA fingerprinting, genotyping, and genetic analysis. As defined by Reshma and Das (2021), molecular markers are constant and inherited variation, that are capable of being quantified or detected by an appropriate method and consequently, can be used to identify the presence of a specific genotype or phenotype. As highlighted by Marwal and Gaur (2020), molecular markers permit the detection of polymorphisms or variations existing among individuals in the population for specific regions of DNA. Diverse forms of genetic markers explore different regions of the genome that have specific evolutionary patterns (Tibayrenc and Ayala, 2017). Intron length polymorphic markers (ILPs) are other EST-based markers that reveal polymorphisms in DNA sequences and they appear unique as they exhibit higher intra-specific polymorphism than other markers. Introns, abundant in eukaryotic genomes, are non-coding sequences in a gene that are transcribed to precursor mRNA but spliced out during mRNA maturation (Gupta *et al*., 2011) thus variations in intron sequences could be used as potential molecular markers as they are highly variable compared to the coding sequences (Bierne *et al*., 2000; Gupta *et al*., 2011). In the view of Wang *et al*., (2005), various polymorphisms could be seen in introns, but intron length polymorphism (ILP) is the most easily recognizable type. Intron markers are thus developed by designing primers in exons flanking the target intron (Yang *et al*., 2007) using an approach called exon-primed intron-crossing PCR (EPIC-PCR) (Wang *et al*., 2005). EST sequences are compared with the genomic sequences of a model plant to determine the intron positions during the development of ILP makers (Gupta *et al*., 2012). ILP has been exploited as a good marker system because they are convenient, reliable molecular markers with high plant interspecies transferability that can be used for the construction of genetic maps and are also neutral, co-dominant, stable and specific since they are tagged to selected genes (Braglia *et al*., 2010).

Advancements in Intron-Based Marker Development

Advancements in the development of plant intron-based marker have been significant, presenting valuable tools for genetic studies and breeding programs. As reported by Grosser *et al.,* (2023), plant mitochondrial introns are utilized for marker development due to conserved exons, allowing for polymorphic markers. Plant genomes consist of nuclear, plastid, and mitochondrial components, each exhibiting distinct patterns of inheritance and evolution. The utilization of genetic markers from these three genomes offers valuable resources for studying inheritance, genetic relationships, and phenotypic contributions. However, the development of universal markers for plant mitochondrial genomes is particularly challenging due to their significant variability in size, gene order, and intergenic sequences. Nevertheless, these mitochondrial genomes remain highly conserved in terms of protein-coding sequences. As such, plant mitochondrial genomes pose challenges due to their variability, but PCR-based intron amplification using conserved sequences has proven effective in developing polymorphic markers (Grosser *et al*., (2023). Plant mitochondrial introns help in distinguishing species and varieties, enhancing genetic studies in plants.

Recent advancements in molecular marker technologies, such as next-generation sequencing, have led to the development of highly informative markers, enhancing plant genotyping and breeding programs. Advancements in sequencing technology have made it possible to conduct thorough sequencing of entire transcriptomes, also known as RNA-seq (Clarke *et al*., 2009). The use of high-throughput transcriptome sequencing offers the advantage of generating extensive transcript sequence datasets, which are valuable for gene discovery and the development of molecular markers. In a study, Ahmadvand *et al*., (2014) created intron-targeting (IT) markers using transcript sequencing data obtained from next-generation sequencing (NGS) technology, specifically from the potato cultivar White Lady. They also examined the applicability of these IT markers in other potato genotypes and investigated their transferability in other *Solanum* species. Furthermore, Zhang *et al*., (2017) utilized next-generation sequencing technology to develop intron targeting (IT) markers that are specific to *Dasypyrum villosum* chromosomes, encompassing the entire genome.

The progress made in the development of PCR-based Intron Polymorphism Markers in Sorghum (Jaikishan, 2015) highlighted the utilization of intron length polymorphisms for various purposes such as genetic diversity analysis, linkage mapping, and comparative genomics studies in sorghum. Rajendrakumar (2015) showed the advancements in the development of intron-based markers involving the exploration of DNA sequence variations such as In-Dels and SNPs derived from ESTs and whole-genome sequences. These markers are valuable tools for marker-assisted selection and the assessment of genetic diversity.

Intron Length Polymorphic Markers in Plant Genetics

Intron Length Polymorphism (ILP) serves as a valuable tool in plant genetics for studying genetic relationships,

inheritance patterns, and phenotypic contributions. One of the main applications of ILP markers in crop species is genetic diversity analysis. ILP markers are valuable tools for interspecific differentiation due to their ability to detect significant differences between species at the DNA level, unaffected by environmental factors (Zebire, 2020). Genetic diversity is a critical aspect of crop improvement and conservation. It refers to the variation of genes within a species, providing the raw material for adaptation to changing environments and the development of new and improved crop varieties. ILP markers can reveal the level of genetic diversity within a crop species by identifying the presence of different alleles at specific loci. This information can be used to assess the genetic variation and relatedness among crop varieties, populations, and even different species. By understanding the genetic diversity present in a crop species, breeders can make informed decisions on how to develop new varieties or conserve the existing ones. Molecular markers, including ILPs, play a crucial role in genetic diversity analysis by aiding in the identification of individual organisms, monitoring genetic integrity, and evaluating biological resources (Nam *et al*., 2020). Xia *et al.,* (2017) reported the use of ILP markers in genetic diversity analysis where they were utilized for phylogenetic analysis in willow samples, highlighting geographic distance correlation. Intron Length Polymorphism (ILP) markers were used to assess the genetic diversity in rice genotypes, aiding in parent selection for breeding programs and establishing the sovereignty of the Bangladeshi rice gene pool (Siddique *et al*., 2017). As reported by Zhang *et al.,* (2017), large-scale ILP markers were developed ILP markers in alfalfa and they aided in genetic diversity analysis by yielding high polymorphism levels (PIC 0.15-0.87) and transferability to various leguminous and non-leguminous species.

ILP markers have been used in diversity studies of wild crop relatives in addition to these uses. Wild crop relatives are closely related species to domestic crops that have important characteristics like high nutritional content, drought tolerance, and disease resistance. The wild crop relatives are essential for crop improvement because these traits can be bred into cultivated crops. ILP markers have been used to study the genetic diversity and relatedness among wild crop relatives, providing important information for breeding programs. Jayaswall *et al*., (2019) reported the aiding of ILP markers in characterizing genetic diversity in wild crop relatives, facilitating trait introgression, varietal identification, and mapping of genes for molecular breeding in Allium species. As reported by Van et al., (2013), ILP markers are utilized in spatial analysis to optimize in situ conservation of plant genetic resources, enhancing diversity studies of wild crop relatives for conservation prioritization.

Another application of ILP markers in crop species is germplasm characterization and identification. Germplasm refers to the collection of genetic resources that are used for breeding and conservation purposes. ILP markers can be used to create a genetic fingerprint of a particular germplasm, allowing for its accurate identification and management. This is especially useful in large germplasm collections where traditional identification methods may be time-consuming and less accurate. ILP markers, derived from exon-flanking introns, are valuable tools for germplasm characterization. They exhibit high polymorphism due to lower selection pressure on introns (Gbotto *et al*., 2022). ILP markers offer a robust co-dominant marker system, providing insights into genetic diversity, facilitating germplasm conservation, and supporting molecular breeding studies Kumar *et al*., (2018). Their application in germplasm characterization contributes significantly to understanding genetic variability and enhancing breeding programs in various crops (Tuvesson et al., 2021). ILP markers, in particular, are expedient in germplasm characterization, genome relationships in millet and non-millet species, and comparative mapping (Muthamilarasan *et al*., 2013, Gupta *et al*., 2011). The validation, cross-genera transferability and hereditary differences consider demonstrated the practicality of these ILP markers in germplasm characterization, genome relationships in millet and non-millet species and comparative mapping. In addition, based on their heterotic group and relationship, they were used for the analysis of genetic diversity and the clustering of maize inbred lines (He *et al*., 2014). ILP markers from starch biosynthesis genes were utilized for the genetic characterization of Indian wheat varieties, showing clear genetic distinctions and potential for germplasm conservation and molecular breeding studies (Sharma *et al*., 2020).

ILP markers have also been used in conservation efforts of endangered crop species. With the increasing loss of biodiversity, many crop species are facing the risk of extinction. ILP markers can be used to assess the genetic diversity of these endangered species, which can aid in developing conservation strategies to preserve their genetic resources. ILP markers are used to evaluate genetic stability in endangered species that are conserved in vitro, guaranteeing that original populations are preserved despite conservation efforts (Sujanani *et al*., 2016). These markers also aid in genuine taxonomic identification, genetic variability analysis, and studies of adaptation, which makes it easier to apply customized conservation strategies for crop species that are in danger of extinction. ILP markers play a critical role in conservation efforts because they protect genetic resources, speed up breeding programs, and increase crop productivity.

Parentage analysis is the process of comparing the DNA profiles of offspring and possible parents to confirm relationships. By identifying and comparing the alleles of

progeny and potential parents, we can determine the maternal and paternal origins of the offspring. Molecular markers enable the analysis of parentage, which is one of the useful tools crop breeders have at their disposal to optimize genetic gain in a breeding program. Resolving the identity of half-sibling progenies and reconstructing the pedigree in outcrossing crops can benefit from it (Norman *et al*., 2018). With the increasing use of hybrid crops, it is essential to have a reliable method of identifying the parentage of a hybrid plant. ILP markers, with their high level of polymorphism, can be used to determine the genetic makeup of a hybrid plant and identify its parentage accurately. This information is crucial for maintaining the quality and purity of hybrid crop varieties. In crops, ILP markers like EST-specific markers can be utilized for parentage analysis, as demonstrated in Rhododendron species and hybrids, aiding in tracking gene flow and confirming parentage (Hui *et al*., 2006). As reported by Xu *et al*., (2008), ILP markers are utilized in parentage analysis of rice crops, aiding in distinguishing between indica and japonica types, providing insights for inter-subspecific hybrid rice breeding. RGA-ILP markers in wheat show potential for parentage analysis in crops, offering efficient polymorphic markers for mapping populations and demonstrating effectiveness in marker development (Zhou *et al*., 2010). Wei *et al*., (2015) however demonstrated that ILP markers, like those based on intron IV length polymorphism in Gene WAG-2, can be utilized for parentage analysis in crops due to their convenience, reliability, and specificity. Similarly, Sharma *et al*., (2015) reported that ILP markers developed in horse gram can be utilized in parentage analysis in crops due to their potential for genetic diversity studies, mapping, and comparative genomics in related legume species.

Implications and Future Directions

The implications of utilizing Intron length polymorphic markers (ILPs) in plant genetic studies are significant for future research in the field. ILPs have proven to be highly informative markers for genetic diversity analysis, parentage analysis, gene mapping, and marker-assisted selection due to their high level of polymorphism within plant genomes. Moving forward, researchers can leverage ILPs to conduct comprehensive studies on population genetics, gene flow, and evolutionary relationships within plant species. Furthermore, the integration of ILPs with other molecular markers like SSRs and SNPs can enhance the accuracy and efficiency of genetic mapping projects. For future directions, exploring novel bioinformatics tools for ILP data analysis and developing standardized protocols for ILP marker development will be crucial to advancing plant genetic studies to new heights. Overall, the use of ILPs holds great promise for unraveling the complex genetic makeup of plants and providing valuable insights into their evolutionary history and adaptation mechanisms. (Saber Mehdizadeh *et al*., 2021).

CONCLUSION

In conclusion, Intron Length Polymorphic (ILP) markers are an effective tool for studying genetic diversity, population dynamics, and evolutionary relationships among plant species in plant genetic studies. Researchers can learn vital information about the genetic composition of plants, which can guide breeding initiatives and conservation efforts, by examining the variation in intron lengths within the genome. Plant populations can be genotyped effectively and economically with ILP markers, enabling high-throughput analysis of several individuals at once. ILP markers are also an effective tool for researching complex traits and locating potential genes for particular traits of interest due to the high degree of polymorphism they exhibit. ILP marker technology has, all things considered, greatly advanced our knowledge of plant genetics and created new opportunities for study in this area. To sum up, ILP markers are highly valuable for genetic diversity research and have multiple uses in crop species. With their faster and more accurate results, these markers have completely changed the field of crop genetics.

REFERENCES

Ahmadvand R, Poczai P, Hajianfar R, Kolics B, Gorji AM, Polgár Z, Taller J (2014). Next generation sequencing based development of intron-targeting markers in tetraploid potato and their transferability to other Solanum species. *Gene, 540*(1): 117-121.

Clarke J, Wu HC, Jayasinghe L, Patel A, Reid S, Bayley H (2009). Continuous base identification for single-molecule nanopore DNA sequencing. Nature Nanotechnology 4: 265–270.

Gbotto AA, Yao NK, Kitavi M, Osama SK, Habimana R, Koffi KK, Bi IA (2022). Genetic characterization of oleaginous bottle gourd (*Lagenaria siceraria*) germplasm from Côte d'Ivoire using agromorphological and molecular markers. Plant Genetic Resources, 20(2): 162-173.

Grosser MR, Sites SK, Murata MM, Lopez Y, Chamusco KC, Love Harriage K, Grosser JW, Graham JH, Gmitter Jr FG, Chase CD (2023). Plant mitochondrial introns as genetic markers-conservation and variation. *Frontiers in Plant Science, 14*: 1116851.

Gupta S, Kumari K, Das J, Lata C, Puranik S, Prasad M (2011). Development and utilization of novel intron length polymorphic markers in foxtail millet (*Setaria italica* (l.) p. beauv.). *Genome, 54*(7): 586-602. https://doi.org/10.1139/g11-020

He C, Liu H, Su S, Lu Y, Luo B, Nie Z, Wu L, Liu D, Zhang X, Rong T, Gao S (2014). Genome-wide identification of candidate phosphate starvation responsive genes and the development of intron length polymorphism markers in maize. *Plant Breeding,*

134(1): *11-16.*. https://doi.org/10.1111/pbr.12230
Jaikishan I, Rajendrakumar P, Madhusudhana R, Elangovan M, Patil JV (2015). Development and utility of PCR-based intron polymorphism markers in sorghum [*Sorghum bicolor* (L.) Moench]. J. Crop Sci. Biotechnol., 18(5): 309-318.
Jayaswall K, Sharma H, Bhandawat A, Sagar R, Yadav VK, Sharma V, Mahajan V, Roy J, Singh M (2019). Development of intron length polymorphic (ILP) markers in onion (*Allium cepa* L.), and their cross-species transferability in garlic (*A. sativum* L.) and wild relatives. Genetic Resources and Crop Evolution, 66: 1379-1388.
Kumar M, Chaudhary V, Sirohi U, Singh MK, Malik S, Naresh RK (2018). Biochemical and molecular markers for characterization of Chrysanthemum germplasm: A review. J. Pharm. Phytochem., 7(5): 2641-2652.
Marwal A, Gaur RK (2020). Molecular markers: tool for genetic analysis. In Animal biotechnology (pp. 353-372). Academic Press.
Mondini L, Noorani A, Pagnotta MA (2009). Assessing plant genetic diversity by molecular tools. Diversity, 1(1): 19-35.
Muthamilarasan ME, Venkata Suresh B, Pandey GA, Kumari KA, Parida SK, Prasad MA (2013). Development of 5123 intron-length polymorphic markers for large-scale genotyping applications in *Foxtail* millet. DNA Research, 21(1): 41-52. https://doi.org/10.1093/dnares/dst039
Nam VT, Hang PL, Linh NN, Ly LH, Hue HT, Ha NH, Hanh HH (2020). Molecular markers for analysis of plant genetic diversity. Vietnam J. Biotechnol., 18(4): 589-608.
Norman PE, Asfaw A, Tongoona PB, Danquah A, Danquah EY, Koeyer DD, Asiedu R (2018). Can parentage analysis facilitate breeding activities in root and tuber crops? *Agriculture, 8*(7): 95.
Rajendrakumar P (2015). Molecular Marker Development Using Bioinformatic Tools. *Sorghum Molecular Breeding*, 179-195.
Reshma RS, Das DN (2021). Molecular markers and its application in animal breeding. In Advances in Animal Genomics (pp. 123-140). Academic Press.
Sharma H, Bhandawat A, Rahim MS, Kumar P, Choudhoury MP, Roy J (2020). Novel intron length polymorphic (ILP) markers from starch biosynthesis genes reveal genetic relationships in Indian wheat varieties and related species. Molecular Biology Reports, 47: 3485-3500.
Sharma V, Rana M, Katoch M, Sharma PK, Ghani M, Rana JC, Sharma TR, Chahota RK (2015). Development of SSR and ILP markers in horsegram (Macrotyloma uniflorum), their characterization, cross-transferability and relevance for mapping. Molecular Breeding, 35: 1-22.
Siddique MA, Khalequzzaman M, Islam MZ, Rashid ESMH, Baktiar MHK, Chowdhury MAZ (2017). Genetic diversity in Aus rice genotypes using ILP markers. Bangladesh Rice J., 20(2): 13-19.
Sujanani S, Ziai MA, Batchelor JC, Roberts DL. (2016). Conservation of endangered plant species using RFID tags. In 2016 Loughborough Antennas & Propagation Conference (LAPC) (pp. 1-3). IEEE.
Tibayrenc M, Ayala FJ (2017). Trypanosoma cruzi and the model of predominant clonal evolution. In American Trypanosomiasis Chagas Disease (pp. 475-495). Elsevier.
Tuvesson SD, Larsson CT, Ordon F (2021). Use of molecular markers for doubled haploid technology: From academia to plant breeding companies. Doubled Haploid Technology: 2: Hot Topics, Apiaceae, Brassicaceae, Solanaceae: 49-72.
Wei H, Fu Y, Arora R (2006). Utilization of intron-flanking est-specific markers in the phylogenetic analysis and parentage identification of *Rhododendron* species and hybrids. J. Am. Soc. Horticultural Sc., 131(6): 814-819.
Wei S, Peng Z, Yang Z (2015). A simple approach based on intron length polymorphism (ILP) for chromosomal localization of Gene WAG-2. Indian J. Gen. Plant Breeding, 75(03): 314-317.
Xia X, Luan LL, Qin G, Yu LF, Wang ZW, Dong WC, Song Y, Qiao Y, Zhang XS, Sang, YL and Yang L (2017). Genome-wide analysis of SSR and ILP markers in trees: diversity profiling, alternate distribution, and applications in duplication. Scientific reports, 7(1): 17902.
XU XM, LIANG KJ, ZHANG SG, Shang W, ZHANG YY, WEI XY, Bei K (2008). Analysis of Indica-Japonica Differentiation in Rice Parents and Derived Lines Using ILP Markers. Agric. Sci. in China, 8(12): 1409-1418.
Zebire DA (2020). Applications of molecular markers in Genetic Diversity Studies of maize. *Nigerian Journal of Biotechnology, 37*(1): 101-108.
Zhang X, Wei X, Xiao J, Yuan C, Wu Y, Cao A, Xing L, Chen P, Zhang S, Wang X, Wang H (2017). Whole genome development of intron targeting (IT) markers specific for *Dasypyrum villosum* chromosomes based on next-generation sequencing technology. Molecular breeding, 37: 1-11.
Zhang Z, Min X, Wang Z, Wang Y, Liu Z, Liu W (2017). Genome-wide development and utilization of novel intron-length polymorphic (ILP) markers in *Medicago sativa*. Molecular breeding, 37: 1-8.
Zhou R, Jia J, Gao L (2010). RGA-ILP, a new type of functional molecular markers in bread wheat. *Euphytica, 172*: 263-273.