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ANALYSIS OF SELECTED MARKER STUDIES ON MINOR POACEAE FORAGES

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Abstract: Pastures are very important for agriculture since the main feeding source of the enormous cattle herd in the world is forage. Pastures comprise plants from several genera of grasses and legumes. In terms of the physical variety, ecology, and economic significance, the Poaceae is among the most significant families of Angiosperms. Regarding stress tolerance, species within this family exhibit a very wide range of variation. In recent years, the importance of using molecular markers in phylogenetic analyses of numerous organisms has increased. The development of genomic technologies and infrastructure has progressed sufficiently for their use in marker- aided selection (MAS) to be studied in several important perennial fodder species. Differences can be directly attributed to minor variations in the genetic code such as phenotype, single sequence repeats (SSRs), and single nucleotide polymorphisms (SNPs). Therefore, breeders can benefit significantly from developing and characterizing new genetic markers. This paper gives a brief analysis of some international studies on some minor Poacea forages.

Keywords: Markers, Forages, Poaceae, Minor

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1. Introduction

The emergence of several genetic markers as a result of molecular biology advancements has transformed our understanding of the structure and evolution of crop genomes. Finding genetic variety in crops provides a chance to comprehend the molecular basis of several biological occurrences (Adhikari et al., 2017). In the last years, the importance of using molecular markers in phylogenetic analyses of numerous organisms has increased. Fast DNA sequencing methods' accessibility and the advancement of reliable statistical analysis techniques gave this discipline a new start. Traditional classification systems of organisms based on morphology have major drawbacks despite still being frequently used. On the other hand, it seems that the classic morphology-based method for phylogenetic investigations can be supplemented via the use of molecular markers, despite recent popularity and the fact that they are not without flaws. Nuclear genome sequencing (NGS) and EST (expressed sequence tag) programs should enhance the number of genes used for phylogenetic analysis of crops, animals and microorganisms. As they exhibit significant commonality across a wide range of organisms, the power of genes involved in an organism's physiology, such as salt tolerance genes, the cell division (cdc) genes, heat shock genes, receptor genes, homeotic genes, etc., should also be investigated (Patwardhan et al., 2014).

Estimation of genetic diversity using a variety of methods is possible by dominant markers (such as DNA Amplification Fingerprinting (DAF), Random Amplified Polymorphic DNA (RAPD), Arbitrarily Primed Polymerase Chain Reaction (APPCR), Amplified Fragment Length Polymorphism (AFLP), Inter-Simple Sequence Repeat (ISSR) and co- dominant markers (such as Restricttion Fragment Length Polymorphism (STSs). New methods are always being developed today. Each method has its own benefits and drawbacks (Idrees and Irshad, 2014).

The development of molecular markers is now moving in new directions owing to public genomic databases, which have also changed the sorts of PCR-based procedures frequently employed in plant science. In addition to the frequently used DNA markers, different approaches have been proposed. Targeted fingerprinting methods include non-identical methodological improvements such as incorporating gene or promoter components into primers using amplified DNA technology applications. Although these semi-random markers have promising qualities, they can cause a collision and heterogeneous issues similar to those discovered with randomly generated fingerprints. Many elements in motion found in plant genomes can also be used to produce fingerprints. By exploiting certain targeted sites, these markers boost genome coverage and generate bands that primarily resemble one another. Another class of recently developed methods takes advantage of the

indicated length polymorphism in different transcript gene families, such as the cytochrome P450 and tubulin genes, to enable interspecies amplification and transferability. In addition, markers can be generated from functional and/or transcribed parts of the genome using different gene targeting methods associated with RNA information (Poczai et al., 2013).

2. Poaceae

Poaceae includes plants such as maize (*Zea mays*), wheat (*Triticum aestivum*), oats (*Avena sativa*), rice (*Oryza sativa*), rye (*Lolium perenne*), sorghum (*Sorghum bicolor*), and barley (*Hordeum vulgare*) that are the main source of nutrition for mankind and grazing animals (Guisinger et al., 2010). In terms of the physical variety, ecology, and economic significance, the Poaceae is among the most significant Angiosperm families. Regarding salinity tolerance, species within this family exhibit a very wide range of variation (Céccoli et al., 2015). Concerning the Poaceae family of cultivated plants, there has been considerable variation in low-temperature tolerance between and within individual species (Tondelli et al., 2011). Poaceae is the most important group of crops susceptible to abiotic stress. Several significant cultivable species exhibit distinct behaviors in response to abiotic stresses: wheat and rice are sensitive, displaying significant production reductions in response to water scarcity and soil salinity, whereas barley has a natural resistance to drought and salt (Landi et al., 2017). The most frequent abiotic stresses that crops can experience in fields are extreme temperatures, droughts, salt, and soil pollution; these changes serve as a general warning to plant productivity and survival, becoming more detrimental when combined. Several biological pathways, including sensing, signaling, transcriptional reprogramming, and protein changes, are activated in plants in response to such conditions. Plant cells typically undergo metabolic and transcriptional reprogramming in response to stress, which results in a global response that ultimately influences plant physiology and development (Chirivì and Betti, 2023).

3. Markers of Poacea Forages

Tropical and subtropical rangeland systems provide core ecosystem services for the welfare of human populations that depend on the availability and quality of fodder resources. Forage species, however, are still largely disregarded in molecular biology studies (Dell'Acqua et al., 2014). Some less- studied minor forage species were extracted from academic databases and summarized here below

3.1. *Themeda triandra*

Themeda triandra is a perennial C4 grass found in many subtropical and tropical regions including Asia, Africa, Australia and Türkiye. It is a source of wild grassy forage in rangeland habitats. As a forage researched primarily for soil remediation, as a competitor for foreign invasive species, and as a vegetation filter, some authors identify it as synonymous with Australian *T. triandra.* It is a member of the *Poaceae* family with a very high protein content. It is an important feed source for cattle and wildlife, particularly in Africa and other dry grasslands (Dell'Acqua et al., 2013) (Figure 1).

Figure 1. Frequently burnt eucalypt forest in Queensland (Avustralia), dominated by kangaroo grass (*Themeda triandra*) (Williams et al., 2022).

Dell'Acqua et al., (2014) presented the first molecular characterization of 71 different genotypes of Themeda. A total 65 of these were from three sites along an old cattle migration route in Kenya. Using AFLP markers gave a basic picture of the genetic variation in Kenya- originated *T. triandra*. To generate the Cluster Analysis unweighted pair group method with arithmetic mean (UPGMA) phylogram,366 polymorphic AFLP loci were employed in total. Based on the Jaccard similarity index, a UPGMA phylogram was generated for the genotypes examined in this study. The distribution of genomic variation was not necessarily consistent with the geographic sample of the population, according to the Principal Component Analysis (PCA) grouping of the genotypes examined in this work. The matrix incompatibility (MI) contribution was determined for each genotype to detect recombination and determine if the individuals used various reproductive techniques. There is statistical correlation between eleven AFLP loci and environmental variation. In Kenyan T. triandra populations, recombination rates, genetic diversity, and population genetic structure were all thoroughly studied.

3.2. *Andropogon* spp.

Andropogon spp. Is an excellent forage for both livestock and wildlife due to its palatability and high biomass which are important characteristics for the selection of monocultures suitable for grassland establishment or extensive grazing practices (USDA- NRSC, 2002) (Figure 2).

Figure 2. Clumps of mature flowering plants of gamba grass (*Andropogon gayanus*) showing a tussocky growth habit (Bebawi et al., 2018).

Akinyemi et al. (2021) studied the genetic diversity of 9 different Andropogon spp. obtained from Ogun State using four microsatellite markers (Xcup63, Phil227562, Xcup14 and CTM59). Using the DNA extraction method of Zymo spinTM technology, genomic DNA was isolated from the succulent leaf portion of Andropogon grass. Each and every locus-population is in Hardy-Weinberg equilibrium. At every locus, there were fewer viable alleles than noted alleles. For all markers of the grass taken into consideration, the determined heterozygosity was higher than expected.

3.3. *Bothriochloa ischaemum*

A bunchgrass with a wide natural distribution spanning Europe, Africa, and Asia is *Bothriochloa ischaemum* (Gabbard and Fowler, 2007). Microsatellite primers for *B. ischaemum* were generated in work by Matakis et al. (2011) to examine the structure of invasive populations in Texas and identify the source of introduction from the native region. They constructed an enhanced genomic library using the biotinylated nucleotide technique, and then used it to isolate and describe ten polymorphic microsatellite markers. Additionally, the primers were examined for amplification in the plants *Dichanthium annulatum*, *Andropogon gerardii*, *Bothriochloa saccharoides*, and *Schizachyrium scoparium var*. scoparium. Researchers oncluded that using microsatellite markers could help understand the path of dissemination, identify the origin of invading, populations, and develop biological control agents for *Bothriochloa ischaemum* invasive populations.

3.4. *Arrhenatherum elatius*

A perennial grass known as *Arrhenatherum elatius* has been introduced worldwide and can be seen growing in various of ecological settings. It is believed that this species was introduced after the cultivation of grasslands increased around the end of the Middle Ages rather than being a native of Central Europe (Michalski et al., 2010). Three taxa from the genus Arrhenatherum, including *A. elatius* subsp. elatius, *A. kotschyi*, and *A. palaestinum*, are listed in Flora of Türkiye (Cabi and Dogan, 2012). Michalski et al. (2010) examined 186 AFLP (amplified fragment length polymorphism) loci in 46 European accessions of A. elatius and discovered a significant level of genetic heterogeneity in this species.

Meng et al. (2011) used 100 inter-simple sequence repeat (ISSR) primers to examine the genetic diversity of 19 different *Arrhenatherum elatius* accessions, of which 11 produced unique amplification results. Out of the 152 bands detected, total 107 were polymorphic. The 19 A. elatius accessions were split into three groups with related circumstances based on the results of the PCA and UPGMA cluster analysis. Among the 19 *A. elatius* accessions under study, genetic distance and geographic distance were correlated.

3.5. *Rhodes grass (Chloris gayana)*

In all tropical and subtropical areas of the world, Rhodes grass is a significant tropical C4 grass. It is a high-yielding and high-quality grass that is either annual or perennial forage. It is also a cover crop to increase soil fertility and decrease soil nematodes (Cook et al., 2005). Diploid and tetraploid varieties of rhodes grass exist. They can vary in characteristics including growth pattern, flowering time, dry mass production, seed production, quality, and tolerance to salinity, frosts, and drought (Loch et al., 2004).

There is intra- and inter-cultivar diversity for the salt tolerance of rhodes grass. Taleisnik et al. (2021) cloned and analyzed plants of the Boma for salt tolerance at the seedling and late stages using AFLP and RAPD amplification patterns. For fingerprinting these clones, both methods were equally effective. Despite AFLP producing more bands, both methods had the same ratio of polymorphic bands, and the fraction presented only intolerant clones. These bands could serve as markers for aided selection, coupled with those only present in sensitive clones.

Negawo et al. (2021) used DArtSeq markers to characterize 104 Rhodes grass genotypes for conservation in the ILRI forage genebank to indicate the collection's population structure and genetic diversity and generate representative subset groups. As a result of the characterization, the average polymorphism information content was between 0.18 and 0.26, and a total of 193,988 SNP markers and 142,522 SilicoDArT markers were formed.

Hierarchical clustering using specific informative markers with a cophenetic correction coefficient of 82% resulted in three and two main clusters with SilicoDArT and SNP markers, respectively. The presence of two primary subpopulations employing both marker types, as revealed by a Bayesian population structure analysis, further demonstrated the collection's substantial genetic diversity. A representative subset of 21 inheritances from

different origins was developed using SNP markers.

In order to develop salt-tolerant clones, Ribotta et al. (2013) assessed the survival percentage under salt stress in 46 diploid and tetraploid clones of rhodesgrass (*Chloris gayana* K.). At 600 mM NaCl, fifteen clones were selected hydroponically. By using survival percentage, salt-tolerant rhodesgrass clones were produced. Using the AFLP approach, genetic diversity in a subset of clones was evaluated. Tetraploid and diploid clones could be distinguished using AFLP. Researchers observed genetic diversity at every ploidy level. Researchs chose Clone parents to produce new synthetic varieties.

3.6. *Agrostis* spp.

From a taxonomic standpoint, Agrostis is regarded as one of the most challenging and complex grass genera (Warnke, 2003). Because of the uniparental mode of transmission, chloroplast markers are helpful for identifying species, studying the evolution of hybrids in plant taxa, and dispersing seeds (Ennos et al., 1999). Within the Agrostis complex and the connected genera of Polypogon, Zapiola et al. (2010) generated 12 novel polymorphic chloroplast microsatellite markers to help identify species that received transgenic pollen.

Agrostis species that are utilized for turf have ambiguous genetic relationships. Between 150 and 200 Agrostis species are thought to exist, and interspecific hybridization is a method that has been used to improve one Agrostis species (Amundsen and Warnke, 2011). Recent research on the chromosomal pairing behavior of inter-specific hybrids has either supported or rejected previously postulated genetic links (Honig et al., 2016).

Using recently developed A. stolonifera microsatellite (SSR) markers, Honig et al. (2016) evaluated the genetic linkages among Agrostis cultivars and accessions. 74 Agrostis cultivars and accessions were utilized to genotype 16 individuals using nuclear SSR (nuSSR) and chloroplast SSR (cpSSR) markers. Agrostis species and cultivars can be distinguished by SSR markers, which are helpful in examining the genetic diversity and connections within the genus Agrostis. The species relationships proposed by Jones in the 1950s were most closely mirrored by genetic relationships based on SSR markers. NuSSR marker-based genetic linkages within the Agrostis species closely matched known pedigree links.

3.7. Timoty (*Phleum pratense* L.)

Phleum pratense L. is a perennial grass species that is cultivated in temperate regions of Europe, North America, and Asia. According to Tanhuanpää et al. (2016), the Nordic countries use timothy as their primary forage grass. The cool-season perennial grass species timothy, which can live in a short growing season and is resistant to frost and ice- encasement, is one of Norway's most significant forage grass species (Kovi et al., 2021). High output, feed quality, and winter survival are the key objectives of timothy breeding. Abiotic and biotic factors also contribute to winter injuries. Low-temperature parasitic fungi are one of the main causes of inadequate overwintering. *Typhula ishikariensis* Imai (syn. *T. idahoensis* Remsb.), speckled snow mold, is one of the most important pathogens in the cold climates of the northern hemisphere (Smith et al., 1989).

To break the forage yield plateau in breeding timothy (*Phleum pratense* L.), molecular markers may be useful (Tanaka et al., 2015). Using bulked-segregant analysis, conducted a study to identify DNA markers linked to timothy's resistance to *Typhula ishikariensis*. The cross of the Japanese sensitive cultivar Nosappu with the Finnish resistant cultivar Tammisto II resulted in a progeny of 161 F1 individuals. With a total of 292 primer combinations, resistant and susceptible bulks of eight individuals in each were examined. Together, these six DNA markers and their associations with resistance account for 15% of the phenotypic diversity in Typhula resistance. One linkage group, made up of four markers, contained a QTL that accounted for 7% of the variation in Typhula resistance.

3.8. *Paspalum* spp.

Paspalum is an important genus in the class *Paniceae*, with a complex taxonomic classification as well as diverse forage, ornamental, and weed commercial value. Different species of these plants have been preserved in germplasm banks and dispersed around the world, mostly for cultivar development and cytogenetic research, due to the great interest shown in many species of this genus. Accurate identification of germplasms and measurement of their variability is essential for their use in breeding programs and their appropriate containment (Cidade et al., 2013).

Silveira et al. (2022) conducted a study to measure a range of genetic parameters and predict yield increases in the P. notatum in-species hybrid population. High genetic variability for the production of fodder was evident in the genetic material under study. According to the analysis, new crosses should include 30N male parents and 132,332,336,437 crosses to improve dry matter production of P. notatum. Parents must be chosen from several groups to maximize genetic heterosis and variety. These parents must also be a part of diallel crosses. For a triat of interest, they constructed divergent groups containing genotypes, each distinctly identical, and allowed the selection of superior parents from each group. Selection os superior P. notatum forage hybrids for pastoral systems can be performed using different analyzes and genetic parameters estimated by REML (residual maximum probability). In plant breeding, multivariate analytics are essential tools.

A total of 214 isolates of Paspalum (177 were sampled from 35 species and 37 unclassified) were included in the study by (Cidade et al., 2013). Seventeen novels of SSR polymorphism loci were discovered for the Paspalum species under investigation. Of the 23 microsatellite primer pairs examined for transferability to other species, 12 (52%) amplified their loci in most species.

3. Conclusion

In recent years, the importance of using molecular markers in phylogenetic analyses of numerous organisms has increased. Genetic diversity, population genetic structure and recombination rates in populations of different species were investigated in detail in studies. Genetic distance and geographic distance were studied in many types of research. Some research also chose Clone parents to produce new synthetic varieties. Chloroplast microsatellite markers helped to identify species that received transgenic pollen. Accurate identification of germplasms and determination of their variability rates is essential for the development of more effective conservation and breeding programs. Adopting a molecular genetics method using microsatellite markers in the initial assessment of germplasms helps identify the species and assess the likelihood of successful hybridization.

Author Contributions

The percentage of the author(s) contributions is present below. All authors reviewed and approved final version of the manuscript.

	B.B.	N.B.
C	50	50
D	50	50
S	50	50
L	50	50
W	50	50
CR	50	50
SR	50	50
PM	50	50
FA	50	50

C=Concept, D= design, S= supervision, L= literature search, W= writing, CR= critical review, SR= submission and revision, PM= project management, FA= funding acquisition.

Conflict of Interest

The authors declared that there is no conflict of interest.

Ethical Consideration

Ethics committee approval was not required for this study because of there was no study on animals or humans.

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