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Genetic Insights into Poaceae Forages: A Review of Current Marker Studies

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ABSTRACT

Forage variety development for diversified environmental conditions may benefit from the use of genomic-based breeding procedures. In today's conditions, molecular markers are used by researchers in this field to track loci and genome regions in crop breeding studies. Although earlier characterization efforts yielded useful information, morphological traits and RAPD markers have limitations when used together for genetic diversity research. Different combinations of methodologies are required for diversified aims to study different forage species at the genetic level and to connect micro level traits to macro level traits.

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Introduction

In the majority of semi-arid tropical nations, the lack of sufficient feed and fodder has been one of the main factors preventing livestock production from meeting an acceptable level (Ponnaiah et al., 2019). One of the key methods for increasing forage productivity is to assess forage crops' adaptation and performance across a range of production systems and settings (Habte et al., 2020). There is a need to introduce new, eco-friendly, and highly productive forage grass species to the market (Villegas et al., 2020). In many locations around the world, agricultural sustainability is threatened by drought and a lack of irrigation supplies. Crop variety development for these conditions may benefit from the use of genomic-based breeding procedures (Singh et al., 2022).

The benefits of combining traditional breeding methods with molecular technologies to generate fodder and turf cultivars have long been recognized by researchers and breeders. Despite this, conventional breeding is still used for the forage and grass cultivars that are currently available. This contrasts with the rise in research papers on the characterisation of germplasm resources using DNA markers and the identification of QTLs for many traits in many species (Roldán-Ruiz & Kölliker, 2010).

In crop breeding efforts, the involvement of molecular markers in research for monitoring loci and genomic areas has become standard. It is known that many of the molecular markers used in current studies are obtained from libraries of genomic DNA or randomly amplified PCR fragments. For marker-assisted breeding, mapping interested genes, and cloning genes by cloning techniques based on mapping, molecular markers are necessary (Hayashi et al., 2004). Phylogenetic study, characterisation of the germplasm, and gene introgression by backcrossing are a few further applications of molecular markers. Also, microsatellites have distinguished themselves as the preferred class of ready-to-use markers for breeding work on plants. Both RAPD (random amplification of polymorphic DNA) and restriction fragment length polymorphism (RFLP) analysis are difficult to scale to high-throughput techniques or transfer between laboratories. Amplified fragment length polymorphisms (AFLPs) and microsatellites are both effective tools for polymorphism identification. Can be evaluated at the stage of selection of molecular marker traits during several rounds of introgressions, quantify general genetic variability, calculating the percentage of a genome from a donor, identifying genes that are phenotypically

related to a particular trait under investigation, and more (Miah et al., 2013).

Poa Pratensis

Kentucky bluegrass (*Poa pratensis* L.) is regarded as a global species occupying a diversified range of various habitats because of its great adaptability, strong spreading capability, and significant expansiveness. Due to its widespread distribution and ease of adaptation to drastically diverse conditions, the species has given rise to a large variety of ecotypes that thrive in a variety of settings (Szenejko et al., 2016).

Poa pratensis is a significant temperate perennial grass species that is grown for both turf and pasture. This species can reproduce at many different and unusual ploidy levels through apomixis, giving rise to several morphologies that are genetically distinct. In the past, a variety of different Kentucky bluegrass cultivars and accessions have been identified based on typical turf performance or physical traits as well as through RAPD markers. Although earlier characterization efforts yielded useful information, morphological traits and RAPD markers have limitations when used together for genetic diversity research (Honig et al., 2010).

Molecular markers and flow cytometry are both required to distinguish between various apomictic offtypes. Sets of markers are required, and cryptic molecular variation must be taken into account for determining how similarity among hybridization progeny and cultivar differentiation may be assessed. For greater genotyping effectiveness, high-throughput genotyping platforms are essential (Bushman et al., 2018).

Total 25 SSR markers were used to genotype 247 Kentucky bluegrass varieties, experimental selections, and collections in the study by Honig et al. (2012). In addition to providing support for a revision or update of the classification system, SSR markers demonstrated a good association between genetic relatedness as determined by molecular markers and the original Kentucky bluegrass categorization system. With the existing set of SSR markers, the majority of cultivars, experimental choices, and collections could be uniquely identified. Individuals' genetic ties, as determined by SSR markers, closely matched established pedigrees.

***Brachiaria* spp.**

A highly significant forage species grown in the tropics is *Brachiaria ruziziensis*. Breeding efforts for *B. ruziziensis* may benefit from the use of genomic techniques to assist in the selection of superior genotypes. The genome of *B. ruziziensis*, however, is completely unknown. Additionally, there aren't many genomic tools, including molecular markers, available to enable *B. ruziziensis* breeding efforts (Silva et al., 2013) (Figure 1).

In contigs with a minimum of 10X coverage, Silva et al. (2013) found almost 85,000 perfect microsatellite loci. To design and synthesize the primers, the scientists have chosen only one fromcollection of 500 microsatellite loci located together with a minimum measurement of 100X. Subsequently tested a subset of 269 primer pairs, 198 of which were polymorphic, on 11 representative *B. ruziziensis* entries. Finding and generating microsatellite markers using genome-assembled Illumina single-ended DNA sequences is remarkably efficient. The markers produced for the genetic analysis and marker-assisted selection of *Brachiaria ruziziensis* can simply be put into practice. Reproductive studies are for species with unknown genomic information that could enjoy the benefits of genomic tools. This method for developing microsatellite markers is promising.

Figure 1. Formation of Brachiaria brizantha pasture in year 3 of agroforestry association with eucalyptus (de Souza et al., 2012)

In 2017, Ondabu et al. (2017) gathered 79 Brachiaria ecotypes from several locations in Kenya and investigated their genetic differences and relationships to 8 commercial variants. 22 markers identified a total of 120 distinct alleles in the 79 ecotypes. In identifying ecotypes with average diversity and polymorphism information richness, markers were quite helpful.

In order to find molecular markers for apospory, Thaikua et al. (2016) used an AFLP linkage map of the apomictic pollen donor of the first apomictic hybrid variety of brachiariagrass ('Mulato'). There were 272 markers in 29 linkage groupings on the map. In linkage group 2, twelve AFLP markers associated with apospory that were closely clustered were found. Using basic interval mapping and composite interval mapping, researchers discovered QTLs (quantitative trait loci) for leaf width, leaf width/length ratio, stem diameter, and percentage of filled seeds. The QTLs linked to significant agronomical features and AFLP markers closely linked to apospory are known to be useful for marker-assisted selection in breeding processes in brachiariagrass.

Bromus

There are approximately 150 C3 grass species in the Bromus genus, which is extensively dispersed in the temperate world. Many of these species lack taxonomical identification (Williams et al., 2011). One significant type of grass, prairie grass (*Bromus catharticus* Vahl), has the potential to be employed in systems for producing both high-quality feed and certified seed for grazing ruminants (Yi et al., 2021). A typical cool-season forage crop, *Bromus catharticus* produces a lot of biomass and grows

quickly in the winter and spring. However, because of the restricted number of genomic resources accessible, its genetic research and breeding have stagnated (Sun et al., 2021).

Based on 15 SCoT primers and 15 ISSR, Safari et al. (2019) assessed interspecifc connections in 90 accessions from 18 Bromus species. SCoT markers performed better in separating the accessions than ISSR markers. Based entirely on DNA molecular markers, the various parts of the Bromus genus split apart. Each species' accession may be distinguished using SCoT markers.

With high-throughput transcriptome sequencing use and pre-confirmed EST-SSR markers for *B. catharticus*, Sun et al. (2021) aimed to produce large amounts of genomic data. A new high-yield prairie grass strain, BCS1103, was used to harvest 11 tissue samples, including leaves, stems and seeds. 52 primer pairs with high polymorphisms were chosen out of 420 synthesized by researchers. To understand genetic diversity and population structure in twenty-four *B. catharticus* accessions from around the world. The values from the phylogenetic study are in agreement with the phenotypic clustering, which divided the investigated accessions into four clades. The Mantel analysis revealed that the genetic component generated the majority of the total phenotypic variation. Significant correlations between genetic information and plant height, stem diameter, leaf width, and biomass yield were observed. The development of a genomic library will assist in future research on *B. catharticus* and its relatives' genetics, taxonomy, and molecular breeding.

Yi et al. (2021) used a SRAP (sequence-related amplified polymorphism) marker to reveal the genetic diversity and structure of 80 fescue grass varieties (*Bromus catharticus*) from almost all over the world. From 47 SRAP primer pairs, 460 reliable bands were amplified, with 345 (75%) polymorphic bands. It was observed that five cluster separations were formed with 80 wild grass entries in PCoA and UPGMA clustering analyses, while the STRUCTURE analysis revealed that the 80 accessions had three genetic memberships.

Festuca

The Poaceae family's largest genus, Festuca, contains more than 600 species that are found worldwide in temperate grassland regions. These species are all adapted to very crowded area and various ecogeographical situations (Cheng et al., 2016). Festuca and its closely related genus Lolium are among the non-cereal grasses that have received the greatest attention from agronomists, evolutionists, and plant breeders. Hexaploid tall fescue and diploid meadow fescue are two crucial forage crops for agriculture that belong to the Festuca genus. *F. rubra* L. (Red fescue) and *F. ovina* L. (sheep fescue), which are used as forage and turf, are other fescues of some significance. Although Festuca species are substantially better resistant to abiotic conditions including drought, heat, and low temperatures, they do not compare favorably to Lolium species in terms of providing animal fodder because Festuca species exhibit poor establishment and relatively lower quality traits (Yamada, 2011).

Tall fescue is widely utilized as a turfgrass and a dominant forage grass in the pastoral and turf industries throughout temperate regions of the world. However, due to the roughness of its leaves, poor capacity for regeneration, and weak stress resistance, tall fescue's application was constrained. Modern pastoral enterprises desired new cultivars because they had greater potential than old cultivars (Lou et al., 2015).

Some of the most significant forage grasses in the entire world are part of the Festuca-Lolium species complex. A number of Festuca-Lolium complex species also exist, although these species are closely related to important cereal crops and share more than one trait with wheat (*Triticum aestivum* L.), barley (*Hordeum vulgare* L.) and other well-researched crops. It is known to have distinctive biological and genomic features. Genetic studies are problematic due to crossover structures and the scarcity of molecular markers. Genomic maps of *F. pratensis* and *L. multiflorum* were analyzed by Bartoš et al. (2011) using Diversity Array Technology (DArT) pointers and the DArtFest array. Genetic maps of *L. multiflorum* and *F. pratensis* showed that each crop contained 530 and 149 DArt markers, respectively, and 20 of these markers were reported to be mapped in both species. The ranking of the markers was done and a comparison with each other was made. A sequencing study between Brachypodium, rice, and *L. multiflorum* was conducted. A total of 96 markers were found to be significantly related to the ability to withstand freezing, and five of these markers had genetic associations with chromosomes 2, 4, and 7. Three genomic regions that were previously identified as being linked to freezing tolerance co-localized with chromosomal segments and QTLs in the population. The current research unequivocally supports the DArTFest array's potential for use in genomic analyses of the Festuca-Lolium complex.

Tall fescue and other outbreeding forage grass cultivars are generally generated by intercrossing several carefully chosen parents using the polycross method. Amini et al. (2018) assessed the use of AFLP molecular markers to maximize genetic variety in a tall fescue polycross breeding program. Two polycrosses of six parental plants with differing levels of genetic variation were developed to assess phenotypic traits and AFLP molecular markers. Six of the highest general combining ability genotypes were used to produce a fifth polycross population. The results demonstrated that parental selection with molecular marker assistance produced superior offspring, suggesting that selection based on molecular marker diversity may be a suitable strategy to enhance tall fescue's first-generation progenies.

Orchardgrass (*Dactylis glomerata* L.)

A perennial forage grass with a wide range of variation is *Dactylis glomerata* L. It is widely farmed throughout the entire temperate and subtropical growing zones of the earth (Peng et al., 2008). Long-lived, cool-season orchardgrass (*Dactylis glomerata* L.) is a fodder grass that is frequently used to make hay. Despite being economically significant, orchardgrass genome research is still in its infancy (Huang et al., 2015).

Xie et al. (2010) used cereal EST-SSRs and orchardgrass SSR markers to examine the diversity and

genetic linkages among 74 orchardgrass accessions in order to assess genetic variability and compare the degree of diversity. A total of 190 polymorphic bands were found, with an average of 6.3 alleles per SSR locus. The significant level of genetic variety was indicated by the average polymorphism rate. Additionally, research suggested that the orchardgrass diversity differentiation core may be located in northern Africa, Europe, or temperate Asia.

By using three different DNA-based methods, RAPD, Inter Simple Sequence Repeat (ISSR), and AFLP markers, Costa et al. (2016) were able to identify genetic differences between the three distinct subspecies of *Dactylis glomerata* and generate genotype fingerprints of the species. In this study, 97 bands were generated by RAPD tests, 40 of which (41.2%) were polymorphic. 54 of the 91 bands that the ISSR primers amplified (or 59.3%) displayed polymorphism. Finally, 100 bands were visible on the AFLP, 92 of which were polymorphic (92%). Researchers came to the conclusion that if the genotypes under study are closely connected. Many DNA-based techniques may be required to analyze variability. In fact, genetic variation from different sources may interact with the potential to be more or less polymorphic, or fusion may occur. The results indicated that AFLP may be the most logical molecular analysis for fingerprinting and evaluating of genetic relationships found among Dactylis glomerata genotypes.

Although orchardgrass it is a perennial feed with a high awareness plant, but rust infections have significantly decreased its quality and yield. Yan et al. (2016) evaluated genetic diversity and marker-trait relationships for rust in 75 orchardgrass accessions. By recruiting 18 EST-SSR and 21 SCOT markers. High genetic diversity was detected in the orchard. There were 164 and 289 total bands for the EST-SSR and SCoT markers, respectively. Results revealed that EST-SSRs are inferior to SCoTs in terms of marker efficiency (8.07). (4.82). Twenty band panels were connected to the rust characteristic, according to an association analysis.

Thinopyrum

Many grass species in Triticeae serve as high-value gene pools in feed and cereal crop breeding programs (Hu et al., 2012a). Many scientists choose the key wheat relative *Thinopyrum elongatum* because it has disease-resistance genes in its E genome. According to some research, the Fusarium head blight and wheat rust resistance genes can be seen on chromosome 7E of Th. Therefore, breeding molecular markers specific to chromosome 7E linked to resistance genes will play a very important mediator role in finding and applying resistant genes in Th. Additionally, it would make a significant contribution to the endeavor to develop wheat types that are disease-resistant (Chen et al., 2013) (Figure 2).

An excellent gene pool for wheat enhancement is *Thinopyrum elongatum*. Through chromosomal modification, genes for resistance to numerous biotic and abiotic stressors were introduced from *Th. elongatum* to wheat. With the help of molecular markers, breeding programs can screen a large number of genotypes for the introduction of foreign chromosomes (Hu et al., 2012b).

SLAF-seq technology has been extensively employed in molecular breeding, system evolution, and germplasm resource discovery because of its high throughput, high accuracy, and low cost. In the study by Chen et al. (2013), a total of 518 particular segments of the 7E chromosome of *Th. elongatum* were effectively amplified based on SLAF-seq. A total of 135 primers were produced according to 135 randomly selected fragments, and 89 specific molecular markers were developed for *Th. elongatum*. These markers have all been found in a range of materials, and it has been established that they are all distinct and stable. The 7E chromosome of *Th. elongatum* can be found using these markers, but they can also be utilized to provide a crucial theoretical and practical foundation for wheat breeding using marker-assisted selection (MAS).

Figure 2. Shallow rooted annual wheat (Triticum aestivum) on the right and deep rooted intermediate wheatgrass (Thinopyrum intermedium) (left). This soil profile was excavated 2.5 at meters dethp (Crews, 2016).

Guo et al. (2016) used 17 SCOT and 10 CDDP markers to investigate the evolutionary links found between these different plant families, 7 entries of Thinopyrum species, 11 entries of Triticum species, and *Hordeum vulgare*. The mean number of alleles for the markers SCoT and CDDP were found to be 8.5 and 6.6, respectively, across species. Results of CDDP markers determined that the genetic linkage was consistent between Thinopyrum spp., Triticum spp. and *H. vulgare* formed by SCOT markers. The findings concluded that Triticum species and Thinopyrum species showed the closest relationships, while *H. vulgare* was somewhat distant from both species. Seven additional markers have been added to understand the introduction of Thinopyrum chromosomes or chromosome fragments into Triticum species. Liu et al. (2018a) were able to detect alien chromatin in a wheat background using 67 Thinopyrum ponticum-specific markers and eight *Th. ponticum*-specific FISH probes designed based on SLAF-seq. By using SLAF-seq, Liu et al. (2018b) generated a physical map of the *Thinopyrum ponticum* chromosome 4Ag, located the blue-grained gene, and produced related specialized markers as well as a FISH probe.

Setaria

The grain, known as foxtail millet (*Setaria italica* L.), is of great importance in terms of food and grazing. It is grown and cultivated for consumption by human and animal populations around the world. Foxtail millet, in terms of its small genome and diploid nature, is fast emerging as a cutting-edge creation for studies of plant architecture, drought tolerance, and C4 photosynthesis in cereal bioenergy products. For this reason, studies on diversification, mapping and functional genomics in this formation should use highly polymorphic, sufficiently costly molecular markers to cover the whole genome (Zhang et al., 2014).

One of the prominent food grain crops in Asia is Setaria italica, which is grown as forage and fodder in America, Australia and Africa. Genome sequencing was received soon. Joint Genomic Institute (JGI) of the US Department of Energy and the Beijing Genomics Institute (BGI), China. Together with proso millet, foxtail millet is the second most produced millet in the world (*Panicum miliaceum*) (Gupta et al., 2012).

The most common method for using heterosis in foxtail millet is to breed male-sterile lines; however, more research needs to be done to understand the genetics of the majority of these lines. In the study of Jun et al. (2013), a highly male-sterile line, Gao146A was investigated. Genetic testing revealed that a single recessive gene was responsible for the predominantly male-sterile phenotype. One gene connected to SSR marker b234 that controls predominantly male sterility was located on chromosome VI using the F2 population obtained from the Gao146A/K103 hybrid. These findings not only helped to speed up the breeding technique known as molecular marker, but they also aided breeding by laying the groundwork for detailed mapping of this extremely male-sterile gene.

Total 28 SSR primer sets were used in the study by Kim et al. (2012) to examine the genetic diversity, population structure, and genetic interactions among 37 accessions of foxtail millet from Korea, China, and Pakistan. 37 foxtail millet accessions had a total of 298 alleles. The SSR diversity of the accession from China was higher than that of the accession from Korea or Pakistan. With a few notable exceptions, a phylogenetic tree built using the un-weighted pair group methods and arithmetic mean methodology identified three main groupings of accessions that were not consistent with geographic distribution patterns. The lack of a link between the accession clusters and their geographic locations suggests that there may have been more than one way for foxtail millet to spread from China to Korea.

Krishna et al. (2018) examined the cross-genome transferability of 26 and 101 simple sequence repeats (SSR) markers from foxtail millet and finger millet, respectively, in eight additional millets. Total cross-genome transferability of other millets was 100% for the 33 finger millet and 2 foxtail millet SSR markers. 101 finger millet SSR markers and 26 foxtail millet SSR markers, respectively, had cross-genome transferabilities ranging from 47.52% to 61.38% and from 30.76% to 69.23%. In comparison to genomic SSR (gSSR) markers, finger millet EST- SSR markers demonstrated a higher level of cross-genome transferability. Additionally, further research on genetic variation analysis, population structure, and germplasm characterisation of millets can be done using SSR markers.

Conclusion

It is highly effective to identify and generate microsatellite markers from genome-assembled Illumina single-end DNA sequences, which is encouraging for species whose breeding programs would benefit from the use of genomic tools but lack sufficient genomic knowledge. If the analyzed genotypes are closely related, more than one DNA-based approach may be needed for the investigation of variability. The breeding program, germplasm collection, and conservation will be greatly simplified as a result.

Researchers and breeders have long known that combining traditional breeding methods with molecular technologies would benefit the production of forage and turf cultivars. Despite this, conventional breeding is still used for the forage and grass cultivars that are currently available. In contrast, there are more and more research articles discussing the characterisation of germplasm resources with the help of DNA markers and the mapping of QTLs for various traits in various species. SLAF-seq technology has been extensively employed in molecular breeding, system evolution, and germplasm resource discovery due to its high throughput, high accuracy, and low cost.

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