Grass Research

REVIEW

Open Access

https://doi.org/10.48130/grares-0024-0017

Grass Research **2024**, 4: e021

Molecular advances in St. Augustinegrass: from DNA markers to genome sequencing

Susmita Gaire, Xingwang Yu and Susana R. Milla-Lewis*

Department of Crop and Soil Sciences, NC State University, Raleigh, NC 27695-7620, USA

* Corresponding author, E-mail: srmilla@ncsu.edu

Abstract

St. Augustinegrass (*Stenotaphrum secundatum* (Walt.) Kuntz) is one of the most important warm-season turfgrass species in the United States. Breeding efforts for this turfgrass have primarily focused on improving turf quality and increasing resistance to various biotic and abiotic stresses, including insects, diseases, drought, cold, and shade. While conventional breeding methods have been widely employed in St. Augustinegrass breeding programs, recent years have seen the integration of molecular tools and techniques such as molecular markers, linkage maps, quantitative trait loci (QTL) mapping, comparative genomics, and transcriptomics. Despite these efforts, genomic resources for St. Augustinegrass are still underdeveloped compared to other economically important crops. The recent establishment of a reference genome for St. Augustinegrass is a major milestone, opening new possibilities for genomics-enabled breeding of this important turfgrass. The use of modern genomic tools like genomic selection and marker-assisted selection (MAS) in breeding programs can enhance selection accuracy, shorten breeding cycles, improve trait incorporation, and significantly boost genetic gains, ultimately leading to the development of superior cultivars that meet industry demands. This review highlights recent advancements in genetics and genomics of St. Augustinegrass and identifies areas that require further research to bridge existing knowledge gaps.

Citation: Gaire S, Yu X, Milla-Lewis SR. 2024. Molecular advances in St. Augustinegrass: from DNA markers to genome sequencing. *Grass Research* 4: e021 https://doi.org/10.48130/grares-0024-0017

Introduction

Among the seven species of the genus *Stenotaphrum, S. secundatum* (Walt.) Kuntze (family Poaceae, subfamily Panicoideae, tribe Paniceae) is the only species commercially used as turfgrass in the United States[1]. Commonly known as St. Augustinegrass, this warm-season turfgrass is native to tropical and subtropical climates. It is predominantly grown in coastal regions and is one of the most adaptable warm-season turfgrasses under shading. St. Augustinegrass is a robust perennial grass known for its dense canopy and aggressive growth, which make it resistant to weed infestation[2]. Additionally, it exhibits relatively lower input requirements (water, fertilizer, pesticides) compared to cool-season turfgrasses like tall fescue (*Festuca arundinacea* Shreb.). Moreover, it thrives well in various soil conditions and high temperatures[2,3]. Despite that, the sensitivity of St. Augustinegrass to low-temperature stress has limited its adaptability and marketability in the transitional climatic zone of the US. Improving its freeze tolerance could expand its geographical distribution and adaptability to colder environments where this species is not currently grown.

St. Augustinegrass has a base chromosome number of nine with five different ploidy levels reported including diploid (2x = 2n = 18), triploid (3x = 2n = 27), aneuploid (2n = 28 – 32), tetraploid (4x = 2n = 36), and hexaploid (6x = 2n = 54)[4,5]. Chromosomal disparities across St. Augustinegrass ploidy levels are closely correlated with adaptive and morphological differences[2,5,6]. The species' genetic diversity is categorized into distinct races and groups contingent on ploidy levels, geographic distribution, and morpho-agronomic traits[4–8]. The existence of genetic variation in St. Augustinegrass is evident across various physiological, morphological, and adaptive traits, encompassing cold, shade, and drought tolerance, disease resistance, turf quality, leaf attributes, growth rate, and stomatal density[2,5,6].

Most of the active breeding endeavors in St. Augustinegrass have been directed toward the improvement of turfgrass quality[9–11] and increasing tolerance to several biotic and abiotic stresses including cold[9,10,12–14], drought[15], shade[16], the southern chinch bug (*Blissus insularis* Barber)[17–20], and gray leaf spot disease (*Pyricularia oryzae* Cavara)[21]. While significant phenotypic variation has been reported in St. Augustinegrass polyploid germplasm[5,15,16,22], their exploitation in breeding programs has been offset by challenges such as diminished pollen viability, sterility concerns, and compromised seed set or development attributed to imbalanced chromosomal compositions, as well as uncertainties regarding the origins of higher-ploidy cultivars[5,22].

Although conventional breeding methods have been widely applied by St. Augustinegrass breeding programs, genomic-based breeding is becoming more popular due to its efficiency in achieving higher genetic gains. However, compared to other popular turfgrass species and other economically important commercial crops, genomic resources for St. Augustinegrass are still in development. Challenges like limited historical sequencing data and molecular markers, inadequate access to a high-quality reference genome, complex regulation of polygenic traits, and funding limitations for St. Augustinegrass research have impeded significant progress in St. Augustinegrass

© The Author(s)

genomics. This review aims to spotlight the recent advancements in genetics and genomics in this species, while also pinpointing areas that require further exploration to bridge knowledge gaps.

Marker development and molecular taxonomy

To date, only clonally propagated commercial cultivars of St. Augustinegrass are available in the market[23]. These cultivars are difficult to differentiate morphologically, particularly under high soil fertility conditions[24]. The first application of molecular markers in St. Augustinegrass was the use of isoenzyme markers for the identification of clonally propagated cultivars. Green et al.[24] were able to distinguish 28 clones of St. Augustinegrass into several groups based on quantitative differences, but no qualitative differences were observed. While the usefulness of isoenzyme variation was demonstrated in this study, the integration of highly reproducible and polymorphic molecular markers was needed to enhance the efficiency of breeding and selection.

The first report on the use of DNA markers in St. Augustinegrass was slightly more than a decade ago by Genovesi et al.[25] who used 144 expressed sequence tags-simple sequence repeats (EST-SSR) for the identification of true hybrids recovered *via* embryo rescue. These hybrids were developed from interploidy crosses between the aneuploid cultivar 'Floratam' and five diploid cultivars. As DNA sequence for St. Augustinegrass was not available at the time, EST-SSR markers derived from buffalograss cDNA sequence data were adopted for the study. These markers not only identified true hybrids but also revealed levels of genetic variation present among analyzed cultivars[25].

After this milestone, several other DNA marker studies were undertaken for molecular taxonomy and genetic diversity exploration[5,8,26–29]. The study by Milla-Lewis et al.[5] was a pioneering effort to comprehensively assess genetic diversity in *Stenotaphrum* germplasm, utilizing both molecular and cytological approaches, primarily focusing on its implications for breeding purposes. Amplified fragment length polymorphism (AFLP) markers were used to fingerprint 40 St. Augustinegrass cultivars and plant introductions, supporting previously known morphological classifications, which were predominantly based on morphological and performance traits[4,6,7]. In this study, grouping accessions by ploidy levels yielded more distinct differentiation than by germplasm type. Furthermore, a clear distinction between *secundatum* and *dimidiatum* accessions as well as between diploid and polyploid accessions was found. The clustering of diploids into two distinct races (Breviflorous and Longicaudatus races) suggested possible dual origins for the species, and the distinct clustering of *S. secundatum* polyploids into the Bitterblue and Floratam groups hinted at either chromosome doubling (followed by chromosome loss), or interspecific hybridization. The authors also pointed out the need for additional investigation into the genome origins of polyploids to identify more usable accessions and to manage potential challenges related to sexual incompatibility.

The results from Milla-Lewis et al.[5] were later supported by Mulkey et al.[8,28]. This study[28] utilized 12 AFLP primer combinations to assess genetic diversity in the University of Florida's St. Augustinegrass germplasm collection, one of the largest in the US, and compared it with publicly available cultivars and plant introductions from the National Plant Germplasm System (NPGS). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) cluster and principal component analysis (PCA) identified two major subgroups that aligned well with ploidy levels, with most diploid accessions in one group and polyploids in the other. Meanwhile, triploid and higher ploidy segregation was less distinct[28]. These studies showed that AFLP markers were valuable tools for evaluating genetic diversity and ploidy levels in *Stenotaphrum* germplasm[5,28]. In a subsequent study, Mulkey et al.[8] used Illumina sequencing data to develop the first set of simple sequence repeat (SSR) markers specifically designed for the species. Ninety-four SSR primer pairs were subsequently used for *Stenotaphrum* germplasm evaluation. Results aligned with previous molecular diversity studies[5,28]. Furthermore, through cluster and PCA, the germplasm collection was classified into six subpopulations. Although the transferability of the markers developed to other turfgrass species was low indicating poor applicability outside of St. Augustinegrass, the study provided valuable insights into genetic relationships and population structure in *Stenotaphrum* and underscored their potential for better parental selection, cultivar development, and strategic germplasm utilization.

Using Roche 454 pyrosequencing technology, Wang et al.[29] also isolated and characterized 33 SSR primer pairs specifically for *Stenotaphrum* Trin grasses, with a particular emphasis on St. Augustinegrass. Of the total sequencing reads produced from this technique, only 4.56% (2,614) of them contained SSRs. The identified primers were deposited in GenBank (accession number: KT036573—KT036605). High genetic diversity among the 18 St. Augustinegrass accessions evaluated was observed with 92% of the scorable bands (161) being polymorphic. These accessions were clustered into three distinct major groups that appeared to be associated with ploidy levels which was consistent with other molecular studies[5,8,28]. The findings of Mulkey et al.[8] and Wang et al.[29] highlight the reliability of high-throughput sequencing for efficient identification and isolation of SSR sequences. The SSR markers developed in these studies could be used for several applications, including assessing genetic diversity within St. Augustinegrass populations, constructing linkage maps, verifying the purity of clonal cultivars, and assisting breeding programs aimed at improving specific traits in St. Augustinegrass through marker-assisted selection (MAS).

The use of molecular markers in St. Augustinegrass has also been reported for cultivar identification[8,26,27]. In the study led by Kimball et al.[26], AFLP markers were used to examine identity preservation in samples of 'Raleigh', a cultivar released publicly by North Carolina State University in the early 1980s. 'Raleigh' samples were collected from production fields across the southern United States and compared to the original stock at NC State. With the analysis of the 143 polymorphic AFLP markers, the study found that samples of 'Palmetto', a modern patented cultivar, maintained higher genetic similarity to its original stock (0.97) compared to samples of 'Raleigh', which exhibited a broader range of similarity values (0.24 to 1). The study concluded that molecular makers can be a valuable tool for protecting clonally propagated turfgrass cultivars. In a subsequent study by Kimball et al.[27], AFLP markers were used to identify the true identity of off-types within 'Captiva', a cultivar

released by the University of Florida in 2007[30], production fields. The study examined 72 samples collected from various sod farms across Florida and compared them to seven reference St. Augustinegrass cultivars, including 'Captiva', 'Bitterblue', 'Floratam', 'Floraverde', 'Palmetto', 'Raleigh', and 'Sapphire'. Results indicated that many off-type samples had the highest genetic similarity to 'Palmetto' (49%), suggesting potential contamination during commercial production of 'Captiva'. Mulkey et al.[8] evaluated 94 SSR markers for varietal identification, as morphological methods have limitations in discriminating closely related materials. SSR markers, being easy to use and highly polymorphic, successfully identified multiple cultivars with unique allele combinations. A set of five SSR markers could uniquely identify 20 out of 22 commercial cultivars offering practical benefits for varietal purity maintenance and breeder selection. These studies underscored the importance of using molecular markers in assessing genetic integrity and identifying contaminants to preserve genetic purity in clonally propagated turfgrass species, benefiting both producers and consumers[8,26,27].

Linkage maps and QTL mapping in St. Augustinegrass

Improvement of complex quantitative traits, which are controlled by multiple genes, can be a challenge in St. Augustinegrass breeding. Traditional breeding methods have limitations in this aspect. Thus, the utilization of advanced marker technologies and statistical approaches is needed. Constructing genetic linkage maps using appropriate populations and markers is critical for quantitative trait loci (QTL) analysis. Linkage mapping requires the creation of genetic maps based on recombination frequencies among markers, enabling the determination of the relative positions of markers in linkage groups. Drawing on these linkage maps, QTL analysis establishes connections between genotypic markers and phenotypic traits. Although QTL mapping studies in St. Augustinegrass have been limited, investigations into this species have yielded a few high-density linkage maps and have played a crucial role in pinpointing QTL and molecular markers linked to both abiotic and biotic stress factors. The primary emphasis has been on responding to environmental stresses such as drought[31–33], freezing temperatures[10], and diseases[34,35], as well as physiological and morphological parameters[10,32,36]. However, these QTL still need to be validated in different populations and environments before they can be applied in marker-assisted selection.

Mulkey[34] constructed the first linkage map for St. Augustinegrass using a combination of 107 AFLP and 36 SSR markers in a pseudo-F_2 population of the cultivar 'Raleigh' × Plant Introduction 410353 (PI 410353). However, the relatively small population size and number of markers utilized resulted in a partial linkage map with low coverage. A higher number of linkage groups (LGs) than the number of chromosomes in each haplotype of St. Augustinegrass (2n = 2x = 18) were obtained: 13 LGs for the Raleigh map and 12 for the PI 410353 map. Using these partial linkage maps, the authors identified four potential QTL associated with gray leaf spot (GLS, causal agent *Pyricularia oryzae* Cavara) resistance; three related to the area under the disease progress curve (AUDPC) and one to the area under the lesion expansion curve (AULEC). However, the limitations in population size and number of markers could pose the issue of overestimating QTL effects. While the initial St. Augustinegrass linkage map required further improvement in its coverage and accuracy for a comprehensive understanding of environmental influences on variances and improved QTL analysis, it laid the groundwork for future genetic mapping efforts.

Kimball et al.[10] constructed the first complete linkage map covering all nine haploid chromosomes of the St. Augustinegrass genome using 160 SSR markers in a pseudo-F_2 mapping population of 'Raleigh' × 'Seville'. The linkage map was used for QTL analysis of field winter survival, laboratory-based freeze tolerance, and turfgrass quality traits. The study identified multiple QTL associated with these traits including overlapping QTL on LG 3 (99.21 cM) for winterkill and spring green-up; on LG 3 (68.57–69.50 cM) for turfgrass quality, turfgrass density, and leaf texture; and on LGs 1 (38.31 cM), 3 (77.70 cM), 6 (49.51 cM), and 9 (34.20 cM) for surviving green tissue and regrowth. Additionally, QTL from the field- and laboratory-based freeze testing co-localized on LG3[10]. This indicated the potential for identifying true candidate genes for freeze tolerance in those regions.

The same population as in Kimball et al.[10] was later used in several other studies for linkage mapping and QTL analysis. Yu et al.[36] developed the first high-density linkage maps for the species using 2,871 genotyping-by-sequencing (GBS)-derived single nucleotide polymorphism (SNPs) markers in combination with 81 SSR markers. This integrated map (named LG1–LG9) covered a total distance of 1,241.7 cM with an average marker distance of 0.4 cM, making it the most comprehensive genetic map for St. Augustinegrass at the time. Maps were also developed for each parental genotype (named RLG1-RLG9 for the 'Raleigh' map and SLG1–SLG9 for the 'Seville' map) and covered a total distance of 1,238.7 cM and 914.2 cM for the 'Raleigh' and 'Seville' maps, respectively. Additionally, these maps were also used to map QTL associated with turfgrass quality traits. A total of 48 potential QTL were identified, with three hot spot regions showing overlap between different traits on LG3 and LG8 of the integrated map. Through annotation, these QTL regions were found to contain genes related to leaf development[36].

The high-density genetic maps by Yu et al.[36] provided a powerful foundation for molecular studies in St. Augustinegrass. A comprehensive multi-year, multi-environment analysis was conducted to detect QTL associated with drought-related traits, including relative water content, chlorophyll content, leaf firing, leaf wilting, percent green cover, and normalized difference vegetative index (NDVI) evaluated in both field and greenhouse settings[31]. The study identified a total of 70 QTL associated with these traits. Overlapping QTL were found in LGs RLG1, RLG4, RLG6, RLG7 and SLG2. Notably, a hotspot region in RLG6 contained five overlapped QTL for multiple traits including leaf wilting, leaf firing, leaf relative water content across both experimental settings. Sequence analysis in overlapped regions in these LGs (RLG1, RLG4, RLG6, RLG7, and SLG2) revealed the presence of nine drought response genes including ZHD and WRKY transcription factors, ethylene-insensitive protein, cold-responsive protein kinase, OBERON-like protein, light-harvesting complex-like protein (OHP2), Magnesium-chelatase subunit (ChlD), Osmotin-like protein and LRR receptor-like serine/threonine-protein kinase (GSO1). This study was further expanded to incorporate QTL mapping of

morphological characteristics to understand their potential correlation with drought tolerance[32]. This was the first study to perform QTL analysis for morphological traits, namely leaf blade width, leaf blade length, canopy density, and shoot growth orientation. Co-localization of QTL associated with morphological and drought-related traits was reported in the study. Two previously reported drought-related QTL[31] for relative water content and percent green cover overlapped with QTL for leaf length and leaf width on SLG3. Meanwhile, no overlapping regions were found between canopy density and shoot growth orientation, and drought-related QTL. However, overlapping QTL for shoot growth orientation and leaf length were found on RLG1, and overlapping QTL for canopy density, leaf length, and leaf width were identified on SLG3. These findings provided evidence of the potential influence of morphological traits on drought stress responses. Within QTL intervals related to drought tolerance and morphological traits, three key genes associated with plant growth and development [Gibberellin 2-beta-dioxygenase (GA2oxs), F-box/LRR-repeat protein (D3), S-adenosylmethionine decarboxylase proenzymes (SAMDCs), two water stress response genes (E3 ubiquitin ligases (PUB22 and PUB23), BAM1 (Beta-amylase)], and two genes contributing to drought tolerance through root system maintenance [GSO1 (Gene controlling primary root growth), Root phototropism protein 2 (RPT2) and Periodic tryptophan protein 2 (PWP2)] were identified.

To address the limitations encountered in Mulkey[34] in mapping QTL for GLS resistance, Yu et al.[35] further expanded the study by increasing the population size to 153 hybrids and using a high number of SNP markers (2,257 and 511 for parents 'Raleigh' and PI 410353, respectively). With these improvements, the authors were able to improve the coverage of both parental linkage maps and detect more putative QTL. Twenty QTL associated with GLS resistance were identified, with three prominent hotspots located in LGs P2 and P5. Notably, two significant QTL, glsp2.3 and glsp5.2, which collectively resulted in a 20.2% reduction in disease incidence, were identified. These results suggested the potential use of these favorable alleles *via* marker-assisted selection in St. Augustinegrass breeding to effectively enhance GLS resistance. However, the lack of available genomic information for St. Augustinegrass at the time limited access to gene information within the QTL intervals. The study resulted in two candidate genes, XM_025948638.1 and XM_004968938.4, that code for β-1,3-glucanases, recognized as pathogenesis-related (PR) proteins, being identified within both glsp2.3 and glsp5.2. While these PR protein genes showed the potential for improving GLS resistance in St. Augustinegrass, a better understanding of their potential role was essential. Additionally, both studies were performed under controlled conditions, and to date, there has been no QTL research to validate these findings in field settings. Further investigations involving multiple environments are essential to elucidate the practical applications of these QTL and underlying genes in breeding programs.

Rockstad et al.[33] developed a new population to validate the results from Yu et al.[31] by crossing breeding lines XSA 10098 and XSA 10127, the most contrasting genotypes in terms of drought response from the 'Raleigh' × 'Seville' population utilized by Kimball et al.[10] and Yu et al.[31]. The study used a draft of the first St. Augustinegrass reference genome[37] for alignment in the SNP calling pipeline, which resulted in the densest linkage map to date using four times as many markers (12,269) compared with 2,952 in Yu et al.[36] and 2,257 in Yu et al.[35]. Among the 24 QTL regions uncovered in this study, 16 were observed to overlap with regions identified in prior studies for drought tolerance[31] and morphological characteristics linked to drought tolerance[32]. These overlapping regions were found on chromosomes 3, 4, 6, and 9. Of particular interest was the co-localization of QTL for percent recovery from drought, percent green cover, leaf wilting, relative water content, and area under the leaf wilting curve in this study with relative water content in Yu et al.[31], which occurred within the QTL region on chromosome 3.

Using the population and linkage map developed by Rockstad et al.[33], Weldt et al.[38] conducted a field evaluation to validate previously identified QTL associated with drought and drought-related traits[31–33]. Weldt et al.[38] addressed the need to validate QTL in different mapping populations and under varying environmental conditions by employing a different mapping population from Yu et al.[31,32] and environments different from those used by Rockstad et al.[33]. The study identified 22 QTL on five linkage groups, with 19 overlapping with QTL from previous studies[31–33] on LGs 1, 2, 4, and 9. Although the same mapping population was used in both the greenhouse evaluation by Rockstad et al.[33] and the field evaluation by Weldt et al.[38], only two QTL in LG1 and LG9 were found to overlap between the studies, highlighting the influence of environmental factors on QTL localization and expression. These QTL could be used in investigating drought avoidance and tolerance traits under field and greenhouse conditions.

Comparative genomics and transcriptomics

Yu et al.[36] used the first high-density linkage map to perform comparative genome analyses between St. Augustinegrass and three other grass species (foxtail millet: *Setaria italica* (L.) P. Beauv., sorghum: *Sorghum bicolor* [L.] Moench, and rice: *Oryza sativa*). This is the only comparative genomics study that has been carried out on St. Augustinegrass to date. The study revealed chromosomal rearrangements and fusion events post-divergence from their common ancestor[36]. St. Augustinegrass and foxtail millet exhibited high synteny and collinearity. However, several inter-chromosomal rearrangements and inversions differentiated their genomes. In sorghum, comparative genomics revealed high collinearity. Notably, an event of nest chromosome fusion was identified, indicating a fusion between sorghum chromosomes Chr8 and Chr9, leading to the formation of an R(S)LG3 in St. Augustinegrass. Using rice as a reference, the study identified three pairs of fused rice chromosomes in St. Augustinegrass, highlighting evolutionary changes among these species. Overall, the study provided insights into the genomic relationships, conservation, and rearrangements between St. Augustinegrass and these model grass species, increasing our understanding of the evolutionary history of the grass family.

The first endeavor to characterize gene expression in St. Augustinegrass at the molecular level was that of Jo et al.[39], who utilized tools and genetic resources from rice to assess the transcriptomic response of St. Augustinegrass to *M. grisea*. Utilizing large-scale EST screening through reverse northern hybridization, 30 rice EST clones, showing differential expression in St. Augustinegrass, were selected and their putative

functions were categorized. The findings revealed a conserved response to *M. grisea* infection between rice and St. Augustinegrass. This study not only provided insights into the identification and characterization of defense-related genes in turfgrass, but also highlighted the potential for leveraging rice genetic resources in understanding the molecular response of St. Augustinegrass to fungal pathogens[39].

Schoonmaker[37] developed the first-ever reference genome for St. Augustinegrass using a combination of PacBio CCS, Illumina, and Hi-C technologies. Two haplotype assemblies were created for the freeze-tolerant diploid cultivar 'Raleigh', with the primary assembly being more complete (465.41 MB in 631 scaffolds) than the secondary one (401.52 MB in 539 scaffolds). Both haplotype assemblies were close to the expected genome size, accounting for 95.2% and 82.1% of the expected haplotype genome size, respectively. Compared to previously published turfgrass assemblies for African bermudagrass (*Cynodon transvaalensis* Burtt Davy)[40] and zoysiagrass (*Zoysia japonica* Steud.)[41], these assemblies had higher contig and scaffold lengths, meeting reference genome quality requirements. A total of 67,805 genes were annotated, and a standardized pipeline was developed for consistent annotation across warm-season turfgrasses. The study also successfully quantified 'Raleigh's' heterozygosity using two haploid assemblies, revealing it to be a hybrid with high levels of heterozygosity. Accurate quantification of heterozygosity in *S. secundatum* had not been previously done[37]. This study not only facilitated the development of crucial tools for future investigations but also provided insights into St. Augustinegrass genetics.

With the availability of a reference genome, Rockstad et al.[33] and Weldt et al.[38] investigated differentially expressed genes (DEGs) in leaves and roots, respectively, under drought stress conditions compared to normal watering, focusing on both tolerant (XSA10098) and sensitive ('Raleigh') genotypes of St. Augustinegrass. In leaves, the drought-sensitive genotype showed changes in the expression of a greater number of genes (either up- or down-regulated) compared to the drought-resistant genotype[33]. Similar results were observed in the roots of St. Augustinegrass[38], indicating a lack of effective regulatory mechanisms such as a homeostatic system to counteract the effects of water deprivation in sensitive genotypes. Stress response-related plant hormone signal transduction pathways such as the abscisic acid metabolic process was upregulated in both leaves[33] and roots[38] and photosynthetic genes were down-regulated in leaves of both genotypes[33]. Tolerant genotypes showed upregulation of secondary metabolite pathways in leaves, while both genotypes had downregulation in roots. Mitogen-activated protein kinase (MAPK) signaling pathway genes showed complex patterns across genotypes and tissue, with downregulation in the sensitive genotype's leaves and mixed regulation in leaves of the tolerant genotype and roots of both genotypes[33,38].

In addition, Rockstad et al.[33] combined leaf transcriptomic data with a QTL mapping study[31,32] for the first time in St. Augustinegrass, which was possible due to the availability of a St. Augustinegrass reference genome[37]. The findings revealed 12 co-localized candidate genes involved in cell wall organization, photorespiration, zinc ion transport, regulation of reactive oxygen species, channel activity, and regulation in response to abiotic stress. In a subsequent study, Weldt et al.[38] identified 21 candidate genes through the integration of root transcriptomics data with prior QTL studies[31–33], revealing a similar pathway and gene involvement. A notable colocalization of DEGs encoding for putative LRR receptor-like serine/threonine-protein kinase, and Cysteine-Rich Peptide Family on chromosome 6 in both leaf and root tissue showed potential functional significance in relation to drought response in St. Augustinegrass[33,38]. These findings are valuable for understanding the mechanisms underlying drought tolerance in St. Augustinegrass and may inform breeding efforts aimed at developing more resilient cultivars by narrowing down the confidence interval of significant QTL and identifying reliable candidate genes within those to be used in MAS.

While the studies summarized here have increased our understanding of the genetic control of some of the most economically important St. Augustinegrass traits and have laid the foundation for the identification of key genes that could be incorporated in breeding programs, most of these studies were conducted in only one population or a single environment, except for the drought studies. Validating the identified QTL in different environments and/or populations is needed before they can be implemented in MAS to increase selection accuracy compared to traditional, phenotype-based selection methods. However, it is imperative to note that MAS may not always be practical, especially for traits governed by multiple minor genes such as stress tolerance-related traits. Integrating genomic selection into turfgrass breeding programs can offer a more suitable alternative with numerous advantages, including increased selection accuracy, accelerated breeding cycles, improved trait incorporation, enhanced genetic gains, and resource optimization. This approach overcomes the challenges associated with MAS for polygenic traits and enables more efficient selection for complex traits like stress tolerance. By leveraging genomic information, breeders can expedite the development of superior turfgrass cultivars that meet the evolving needs of the industry and end-users. Furthermore, the availability of the recently generated reference genome will be instrumental in supporting turfgrass breeding research as it facilitates accurate mapping of DNA markers like SNPs in linkage maps, a crucial step in identifying QTL controlling traits of interest. Furthermore, a reference genome enables the integration of meta-QTL[42], genome-wide association (GWAS) and -omics studies, as well as cross-species comparative genomic and transcriptomic analyses. Integration of these approaches in discovering genomic regions and candidate genes associated with traits of interest can expand the molecular tools available for improved selection accuracy and increased efficiency in the breeding pipeline, marking the beginning of the genomics-enabled breeding era for St. Augustinegrass.

Author contributions

The authors confirm contribution to the paper as follows: study conception and design: Milla-Lewis SR, Gaire S, Yu X; data collection: Gaire S; analysis and interpretation of results: Gaire S; draft manuscript preparation: Milla-Lewis SR, Gaire S, Yu X. All authors reviewed the results and approved the final version of the manuscript.

Data availability

Data sharing not applicable to this article as no datasets were generated or analyzed during the current study.

Acknowledgments

The authors wish to thank The North Carolina State University Center for Turfgrass Environmental Research and Education for funding provided to support the PhD student working on this project.

Conflict of interest

The authors declare they have no conflict of interest. Susana R. Milla-Lewis is the Editorial Board member of *Grass Research* who was blinded from reviewing or making decisions on the manuscript. The article was subject to the journal's standard procedures, with peer-review handled independently of this Editorial Board member and the research groups.

Dates

Received 30 June 2024; Revised 9 August 2024; Accepted 13 August 2024; Published online 26 September 2024

References

1. Christians NE, Patton AJ, Law QD. 2016. Warm-season grasses. In *Fundamentals of Turfgrass Management*, fifth edition. USA: John Wiley & Sons. pp. 75–95. doi: 10.1002/9781119308867.ch4
2. Busey P. 2003. St. Augustinegrass, *Stenotaphrum secundatum* (Walt.) Kuntze. *Biology, Breeding, and Genetics of Turfgrasses*, eds Casler MD, Duncan RR. Hoboken, NJ: John Wiley & Sons, Inc. pp. 309–30
3. DiPaola JM, Beard JB. 1992. Physiological effects of temperature stress. In *Turfgrass*, volume 32, eds Waddington DV, Carrow RN, Shearman RC. pp. 231–67. doi: 10.2134/agronmonogr32.c7
4. Busey P, Broschat TK, Center BJ. 1982. Classification of St. Augustinegrass. *Crop Science* 22(3):469–73
5. Milla-Lewis SR, Zuleta MC, Van Esbroeck GA, Quesenberry KH, Kenworthy KE. 2013. Cytological and molecular characterization of genetic diversity in *Stenotaphrum*. *Crop Science* 53(1):296–308
6. Busey P. 1995. Genetic diversity and vulnerability of St. Augustinegrass. *Crop Science* 35(2):322–27
7. Busey P. 1986. Morphological Identification of St. Augustinegrass cultivars. *Crop Science* 26(1):28–32
8. Mulkey SE, Zuleta MC, Keebler JE, Schaff JE, Milla-Lewis SR. 2014. Development and characterization of simple sequence repeat markers for St. Augustinegrass. *Crop Science* 54(1):401–12
9. Kimball JA, Isleib TG, Reynolds WC, Zuleta MC, Milla-Lewis SR. 2016. Combining ability for winter survival and turf quality traits in St. Augustinegrass. *HortScience* 51(7):810–15
10. Kimball JA, Tuong TD, Arellano C, Livingston DP, Milla-Lewis SR. 2018. Linkage analysis and identification of quantitative trait loci associated with freeze tolerance and turf quality traits in St. Augustinegrass. *Molecular Breeding* 38:67
11. Gouveia BT, Rios EF, Nunes JAR, Gezan SA, Munoz PR, et al. 2021. Multispecies genotype × environment interaction for turfgrass quality in five turfgrass breeding programs in the southeastern United States. *Crop Science* 61(5):3080–96
12. Li R, Qu R, Bruneau AH, Livingston DP. 2010. Selection for freezing tolerance in St. Augustinegrass through somaclonal variation and germplasm evaluation. *Plant Breeding* 129(4):417–21
13. Moseley D, Patton A, Trappe J. 2012. Winter hardiness of thirty St. Augustinegrass genotypes. In *Arkansas Turfgrass Report 2010*. pp. 79–84. https://core.ac.uk/download/pdf/215462323.pdf
14. Moseley DO, Trappe JM, Milla-Lewis SR, Chandra A, Kenworthy KE, et al. 2021. Characterizing the growth and winter survival of commercially available and experimental genotypes of St. Augustinegrass. *Crop Science* 61(5):3097–109
15. Meeks M, Chandra A. 2020. Drought response and minimal water requirements of diploid and interploid St. Augustinegrass under progressive drought stress. *Crop Science* 60(2):1048–63
16. Meeks M, Chandra A. 2021. Performance of diploid and interploid hybrids of St. Augustinegrass under moderate shade. *Crop Science* 61(5):3719–33
17. Busey P. 1990. Polyploid stenotaphrum germplasm: resistance to the polyploid damaging population southern chinch bug. *Crop Science* 30(3):588–93
18. Busey P, Zaenker EI. 1992. Resistance bioassay from southern chinch bug (Heteroptera: Lygaeidae) excreta. *Journal of Economic Entomology* 85(5):2032–38
19. Youngs KM, Milla-Lewis SR, Brandenburg RL, Cardoza YJ. 2014. St. Augustinegrass germplasm resistant to *Blissus insularis* (Hemiptera: Blissidae). *Journal of Economic Entomology* 107(4):1688–94
20. Milla-Lewis SR, Youngs KM, Arrellano C, Cardoza YJ. 2017. Tolerance in St. Augustinegrass germplasm against *Blissus insularis* Barber (Hemiptera: Blissidae). *Crop Science* 57(S1):S-26–S-36
21. Carbajal EM, Ma B, Zuleta MC, Reynolds WC, Arellano C, et al. 2021. Identification of sources of resistance to gray leaf spot in *Stenotaphrum* germplasm. *Crop Science* 61(5):3069–79
22. Moser LE, Burson BL, Sollenberger LE. 2004. Warm-season (C_4) grass overview. In *Warm-Season (C_4) Grasses*, eds Moser LE, Burson BL, Sollenberger LE. Volume 45. USA: American Society of Agronomy, Crop Science Society of America, Soil Science Society of America. pp. 1–14. doi: 10.2134/agronmonogr45.c1
23. Hanna W, Raymer P, Schwartz B. 2013. Warm-season grasses: Biology and breeding. In *Turfgrass: Biology, Use, and Management*, eds. Stier JC, Horgan BP, Bonos SA. Volume 56. USA: American Society of Agronomy, Soil Science Society of America, Crop Science Society of America, Inc. pp. 543–90. doi: 10.2134/agronmonogr56.c16
24. Green RL, Dudeck AE, Hannah LC, Smith RL. 1981. Isoenzyme polymorphism in St. Augustinegrass. *Crop Science* 21(5):778–82
25. Genovesi AD, Jessup RW, Engelke MC, Burson BL. 2009. Interploid St. Augustinegrass [*Stenotaphrum secundatum* (Walt.) Kuntze] hybrids recovered by embryo rescue. *In Vitro Cellular & Developmental Biology - Plant* 45:659– 66
26. Kimball JA, Zuleta MC, Martin MC, Kenworthy KE, Chandra A, et al. 2012. Assessment of molecular variation within 'Raleigh' St. Augustinegrass using amplified fragment length polymorphism markers. *HortScience* 47(7):839–44
27. Kimball JA, Zuleta CM, Kenworthy KE, Lu H, Milla-Lewis SR. 2013. Molecular markers enable the identification of contaminants in production fields of 'Captiva' St. Augustinegrass. *Proc. The 12th International Turfgrass Research Conference, Beijing, 2013*, pp. 267–73. Beijing: International Turfgrass Society.
28. Mulkey SE, Zuleta MC, Van Esbroeck GA, Lu H, Kenworthy KE, et al. 2013. Genetic analysis of a St. Augustinegrass germplasm collection using AFLP markers. *Proc. The 12th International Turfgrass Research Conference, Beijing, 2013*, pp. 281–91. Beijing: International Turfgrass Society.
29. Wang Z, Raymer P, Chen Z. 2017. Isolation and characterization of microsatellite markers for *Stenotaphrum* Trin. using 454 sequencing technology. *HortScience* 52(1):16–19
30. Trenholm LE, Kenworthy K. 2009. 'Captiva' St. Augustinegrass. *Report. ENH1137*. Environmental Horticulture Department, Florida Cooperative Extension Service, Institute of Food and Agricultural Sciences, University of Florida, US. https://sodsolutionspro.com/wp-content/uploads/2020/01/Captiva-General-Captiva_Research_Overview.pdf
31. Yu X, Brown JM, Graham SE, Carbajal EM, Zuleta MC, et al. 2019. Detection of quantitative trait loci associated with drought tolerance in St. Augustinegrass. *PLoS One* 14(10):e0224620
32. Yu X, Lara NAH, Carbajal EM, Milla-Lewis SR. 2022. QTL mapping of morphological characteristics that correlated to drought tolerance in St. Augustinegrass. *PLoS One* 17(5):e0268004

33. Rockstad GBG, Yu X, Gesteira GDS, Gaire S, Dickey A, et al. 2024. Integration of QTL and Transcriptome studies reveals candidate genes for water stress response in St. Augustinegrass. *BMC Biology* 1:under review
34. Mulkey SE. 2013. *SSR Development and mapping of gray leaf spot resistance genes in St. Augustinegrass*. Thesis. North Carolina State University, USA.
35. Yu X, Mulkey SE, Zuleta MC, Arellano C, Ma B, et al. 2020. Quantitative trait loci associated with gray leaf spot resistance in St. Augustinegrass. *Plant Disease* 104(11):2799–806
36. Yu X, Kimball JA, Milla-Lewis SR. 2018. High density genetic maps of St. Augustinegrass and applications to comparative genomic analysis and QTL mapping for turf quality traits. *BMC Plant Biology* 18:346
37. Schoonmaker A. 2023. *A whole-genome assembly of St. Augustinegrass and visualizing diversity within the species*. Thesis. North Carolina State University, USA.
38. Weldt CE. 2024. Integration of multi-omics approaches approaches reveals candidate genes for drought stress in St. Augustinegrass (*Stenotaphrum secundatum*). Thesis. North Carolina State University, Raleigh, North Carolina. www.lib.ncsu.edu/resolver/1840.20/41994
39. Jo YK, Wang GL, Boehm MJ. 2007. Expression analysis of rice defense-related genes in turfgrass in response to *Magnaporthe grisea*. *Phytopathology* 97(2):170–78
40. Cui F, Taier G, Li M, Dai X, Hang N, et al. 2021. The genome of the warm-season turfgrass African bermudagrass (*Cynodon transvaalensis*). *Horticulture Research* 8:93
41. Tanaka H, Hirakawa H, Kosugi S, Nakayama S, Ono A, et al. 2016. Sequencing and comparative analyses of the genomes of zoysiagrasses. *DNA Research* 23(2):171–80
42. Goffinet B, Gerber S. 2000. Quantitative trait loci: a meta-analysis. *Genetics* 155(1):463–73

Copyright: © 2024 by the author(s). Published by Maximum Academic Press, Fayetteville, GA. This article is an open access article distributed under Creative Commons Attribution License (CC BY 4.0), visit https://creativecommons.org/licenses/by/4.0/.