Analyzing genetic diversity and molecular characteristics of wild centipedegrass using sequence-related amplified polymorphism (SRAP) markers (#83622)

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Analyzing genetic diversity and molecular characteristics of wild centipedegrass using sequence-related amplified polymorphism (SRAP) markers

Xiaoyun Wang [1], **Wenlong Gou** [2], **Ting Wang** [1], **Yanli Xiong** [1], **Yi Xiong** [1], **Qingqing Yu** [1], **Zhixiao Dong** [1], **Xiao Ma** [1], **Nanqing Liu** [Corresp., 3], **Junming Zhao** [Corresp. 1]

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Abstract

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Keywords: *Eremochloa ophiuroides*; SRAP; genetic diversity; phenotype

Introduction

Centipedegrass [*Eremochloa ophiuroides* (Munro) Hack.] is a perennial warm-season diploid grass species (2n = 2x = 18) that belongs to the genus *Eremochloa* in the family Poaceae. Centipedegrass originated in southwest China, and the wild population is reportedly mainly distributed in the southern Yangtze River region of China (Hanna & Burton, 1978; He *et al*., 2022). With beautiful leaves, low plant height, drought and barren tolerance, high coverage rate, and strong disease resistance (Cai *et al*., 2022; Li *et al*., 2020), the centipedegrass is a relatively ideal broad-leaved grass species, suitable for building sports and leisure lawn, requiring low maintenance and management. In addition, it can consolidate soil, protect slopes and embankments, and prevent soil and water loss, which plays an important role in slope vegetation restoration (Islam & Hirata, 2010; Liu *et al*., 2008). Therefore, centipedegrass is a pioneer plant for slope ecological restoration. Although centipedegrass is widely distributed in China, with diverse populations and great potential for development, there are few varieties adapted to specific regional climates, which poses a real challenge to the demand for new centipedegrass varieties with long green periods, high overwintering rates, and adaptation to climate change in non-local environments.

Evaluation of genetic diversity in germplasm resources can provide useful information for plant breeding programs (Gawali *et al*., 200). Analysis of the genetic variation in various markers such as morphology, agronomic traits and DNA molecular markers showed significant differences between different accessions and populations (Xuan *et al*., 2005; Zhao *et al*., 2011; Milla-Lewis *et al*., 2012). Compared with other biochemical markers, DNA molecular markers have superior characteristics, such as higher polymorphism, more accurate experimental results, and independence from environmental conditions and developmental stages (Massa *et al*., 2001). Furthermore, they represent a robust and quick approach to detecting the genetic variability of germplasm. Over the years, several molecular markers like amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and inter-simple sequence repeat (ISSR) have been used to elucidate the genetic diversity of centipedegrass accessions (Xuan *et al*., 2005; Zhao *et al*., 2011; Milla-Lewis *et al*., 2012; Massa *et al*., 2001). Sequence-related amplified polymorphism (SRAP) is a new PCR-based approach whereby two sets of primers are designed based on the G and C contents in gene exons to amplify the open reading frame (Li & Quiros, 2001; Robarts & Wolfe, 2014). Compared with other common dominant markers, it is easier to operate, low-cost and more functional. Therefore, in recent years, SRAP molecular marker technology has been widely used for the study of genetic diversity in a large number of grass species, such as *Russian Alfalfa* (Shamustakimova *et al*., 2021), *Buchloe dactyloides* (Wu *et al*., 2019), *Dactylis glomerata* (Zeng *et al*., 2008).

Few studies have hitherto used SRAP to explore the genetic diversity of centipedegrass accessions. This study combined SRAP molecular markers with the seven morphological indexes to reveal the genetic and morphological diversity of 23 centipedegrass accessions. This study aimed to reveal the population genetic structure of these materials at the molecular level. Besides, morphological diversity analysis was conducted to obtain more comprehensive information, which is of great significance for preserving valuable genetic resources, selecting high-quality germplasm resources and developing new varieties.

Materials and Methods

Plant Samples and DNA Extraction

A total of 23 wild centipedegrass accessions were collected in this study collec from Sichuan province (n = 9), Chongqing municipality (n = 6), abroad (n = 1), and other parts of China (n = 7) (Table S1, Fig. S1). In early May 2016, seven morphological traits were measured and scored in the experimental field of Hanchang town, Chengdu city in China (30°35′24″N, 103°31′48″E), which were erect branch leaf length (EBLL), erect branch leaf width (EBLW), stolon leaf length (SLL), stolon leaf width (SLW), stolon internode length (SIL), stolon internode diameter (SLD), and grass height (GLH) (Table S2). We divided the 23 accessions into three groups according to their geographical origins: Sichuan (9 accessions), Chongqing (6 accessions), and Other areas (8 accessions). Dispersed geographical groups with few individuals were classified into the same group.

Genomic DNA was extracted using a plant Genomic DNA Extraction Kit (DP305, Beijing Tiangen). The concentration of DNA was detected by ultramicro spectrophotometer. Completely tested DNA samples were diluted to 10 ng/μL with sterile ddH_2O and stored at -20℃ for PCR amplification.

SRAP Analysis

A total of 215 pairs of SRAP primers were randomly combined to screen polymorphic primers for 23 wild centipedegrass accessions. SRAP amplification system: 15 μL SRAP reaction system: DNA template 3 μL (10 ng μL^{-1}), MIX 7.5 μL (dNTP 240 μmol L^{-1}, Taq enzyme 1.0 U μL^{-1}, Mg^{2+} 2.5 mmol L^{-1}), upstream and downstream primer 0.3 μL (10 μmol L^{-1}) each, ddH_2O 3.6 μL, and Taq enzyme 0.3 μL. The SRAP-PCR reaction was performed as follows: predenaturation at 94℃ for 5 min, 5 cycles of denaturation at 94℃ for 1 min, annealing at 35℃ for 1 min, stretching at 72℃ for 1 min, 35 cycles of denaturation at 94℃ for 1 min, annealing at 50℃ for 1 minute, 72℃ for 1 minute, final extension at 72℃ for 10 minutes and storage at 4°C. The PCR products were

separated by 6% modified polyacrylamide gel and detected by silver staining. Gel clear photographs were used for the following analysis.

Data Analysis

The polymorphic bands were statistically analyzed according to the electrophoresis results. The presence and absence of stripes were recorded as 1 and 0, respectively. Finally, a (0, 1) matrix was generated for statistical software analysis. The number of polymorphic bands (NPB), percentage of polymorphic bands (PPB), marker index (MI) and resolution (RP) were calculated to evaluate the ability of SRAP primers to identify marker differences. PIC was used to evaluate the value of markers for detecting population polymorphism. PIC was calculated by the following formula:

$$PIC = 1 - \sum P_i^2$$

Where Pi is the frequency for the i th microsatellite allele (Riek *et al*., 2001). The GenAlex 6.51 procedure (Peakall, 2012) was used to estimate the effective number of alleles (Ne), Shannon information index (I) and pairwise population PhiPT values (Fst) among the geographical groups. At the same time, principal coordinates analysis (PCoA) was used to analyze the information quality of specific SRAP primers. In addition, NTSYS-pc software was used for cluster analysis of the unweighted pair group method with arithmetic mean (UPGMA), and a tree diagram was generated. The relationship between morphological indexes, climatic data and genetic similarity coefficients of all germplasms was determined by the Mantel test (Zeller Katherine *et al*., 2016). Otherwise, we further evaluated the genetic structure of the population of 23 germplasm resources using the STRUCTURE 2.3.3 software (Pritchard *et al*., 2000), with population K set to 1 - 10. The number of iterations for the burn-in and post-burn periods was set to 10^4 and 10^5 for the Markov chain Monte Carlo simulations. Then the online program was used to determine the optimal K value (Dent A & Bridgett M, 2012).

Results

Primer polymorphism analysis

55 pairs of qualified primers were screened from 215 pairs of primers, and the polymorphism of 23 wild centipedegrass accessions germplasm resources was evaluated. The results showed that the number of reliable bands amplified by each primer pair was 7 (M14E07) - 23 (M01E07), and a total of 919 reliable bands were amplified. The polymorphic bands per primer pair ranged from 16.67% (M07E07) to 90% (M01E20 and M17E10), with an average of 65.8%. The polymorphism and recognition ability of primers were evaluated by PIC, MI and RP. The average PIC value was

0.228, and the PIC value of primer M12E19 was the highest (0.312). The average MI and RP values were 1.85 and 5.40, respectively, indicating the high utility of the primers.

Clustering, PCoA, and Population Structure Analysis

Based on the (0, 1) matrix, UPGMA analysis showed that all accessions could be divided into two clusters (Fig. S3). Cluster I was mainly from Chongqing and other areas, and the cluster II was mainly from Sichuan. Through principal coordinates analysis, another clustering of 23 wild centipedegrass accessions was performed to generate a scatter plot (Fig. 1). The results showed that PCoA divided 23 accessions into two clusters. The molecular variation explained by principal coordinate 1 was 14.31%, which was roughly the same as the result of UPGMA tree. A tree map was constructed based on morphological trait data, and all accessions could be divided into two groups at an average distance of 30.399, indicating that they could be grouped independently regardless of geographic distribution (Fig. S4). The population structure of 23 wild centipedegrass germplasms was analyzed by the Bayesian method. When the Evanno method was performed, the optimal ΔK was 2 (Fig. S5). Accordingly, the optimal number of subpopulations in this study was two (namely, two genetic members) (Table S4 and Fig. 1). Assuming that the accessions with a Q value more than 0.8 were "pure" (Forsberg *et al*., 2014), 69.56 % of the germplasm was attributed to the pure subgroup. The proportion of mixed germplasm resources in Sichuan was the largest (Table S5).

Genetic Structure of the Inferred Geographic Groups and Mantel Analysis

The 23 wild centipedegrass accessions could be divided into three categories: Sichuan, Chongqing and other areas. It was found that Sichuan had the highest genetic diversity (He = 0.201, I = 0.312) (Table 2). The analysis of molecular variance (AMOVA) showed that genetic variation within geo-groups accounted for 88% of the total variation, and the Fst among geo-groups was 0.115, indicating a moderate degree of genetic differentiation among geo-groups (Table 3). The genetic distance between the three geographic groups was evaluated. We found that the differentiation between Chongqing and Sichuan was the lowest (Fst = 0.051) (Table 4).

Mental analysis showed no correlation between the genetic and morphological distance matrices (r = -0.0003, p = 0.5093). When the genetic distance matrix correlated with climate factors, BIO14 (precipitation in the driest month) (r = 0.2513, p = 0.0063), BIO15 (precipitation seasonality) (r = 0.2623, p = 0.0434) and BIO17 (precipitation in the arid region) (r = 0.2354, p = 0.0141) were

highly correlated with genetic distance (Fig. 2, Table S6). At the same time, a correlation was observed between geographical distance and genetic matrix ($r = 0.385352$, $p = 0.000140$).

Discussion

Genetic polymorphism and identification ability of SRAP prime

Compared with other molecular markers, SRAP has the advantages of simplicity, high efficiency, high yield and good repeatability (Budak *et al*., 2004; Gao *et al*., 2020; Li *et al*., 2019). In the present study, 55 SRAP markers were used to evaluate the genetic diversity of 23 wild centipedegrass accessions. Using 55 SRAP markers, 919 scorable fragments were obtained with an average of 16.7 fragments per marker, higher than reported in a study by Zheng *et al*. (2017) in 80 bermudagrass accessions (13.08 fragments per marker) but lower than detected by Yuan *et al*. (2018) in 73 Kentucky bluegrass accessions (18.6 fragments per marker). This finding indicates that the primers screened in this study have very good practicability in the study of genetic diversity. Among the 919 fragments, 606 polymorphic bands were found, higher than in an RAPD study (42.41%) by Xuan *et al*.(2005). This finding indicated that centipedegrass germplasm has high genetic diversity.

It is widely acknowledged that the primer efficiency index represents the overall utility of a specific primer during the identification of many accessions; higher values are associated with higher efficiency and amount of information on primers. In this study, the average MI (1.85) and RP (5.40) values of primers were higher than the SRAP and EST-SSR markers in prairie grass (MI = 1.348, RP = 1.897 and MI = 0.67, RP = 1.14) (Yi *et al*., 2021; Sun *et al*., 2021). Besides, the primer pairs M11E01 (MI = 4.58, RP = 9.91) and M11E09 (MI = 3.74, RP = 9.39) had the highest MI and RP values, indicating that these primers had high genetic identification values for centipedegrass germplasm. Besides, the average PIC value of SRAP markers was 0.228, indicating the high utility of selected primers.

Genetic Diversity of Centipedegrass

In this study, the overall genetic diversity of centipedegrass was higher (He = 0.165) than the average value (0.104) of *Hemarthria compressa* (Huang *et al*., 2012), since the accessions collected in the latter study were concentrated in Yunnan-Guizhou-Sichuan region, the geographical distribution range is limited. Xuan *et al*. (2005) used RAPD to study the genetic diversity of centipedegrass and reported an average value lower than in the present study (0.04 vs. 0.165), which may be attributed to the collection of resources from only five provinces, and these provinces were from the southeast region, which led to low genetic diversity. In the present study,

the 23 wild centipedegrass accessions were collected from a wide geographical distribution, which accounted for their higher genetic diversity. Accordingly, the high genetic diversity of centipedegrass germplasm may be related to its geographical distribution and biological characteristics. Indeed, centipedegrass is a perennial cross-pollination plant with self-incompatibility (Hanna & Burton, 1978). Furthermore, its wide geographical distribution accounts for its adaptability to different ecological environments. In addition, the seed-setting rate of wild centipedegrass germplasm is low. Accordingly, the characteristic of stolon asexual reproduction derived from long-term adaptation and evolution can help maintain the population's genetic diversity (Hanna, 1995; Liu *et al*., 2003).

Genetic differentiation of populations caused by geographical isolation and climatic differences

The clustering results showed that there were significant differences between the Sichuan population and the non-Sichuan population, and other materials except Sichuan were clustered into one group. The reason for this result may be due to human factors, that is, the species resources in a certain area are brought to another place to grow, and then the gene exchange occurs, which is consistent with the research results of *Elymus nutans*(Chen *et al*., 2009). Our AMOVA results showed a certain degree of genetic differentiation among different geographic populations (Fst = 0.115), which was higher than previous studies (0.0643) (Susana R *et al*., 2012), it is also higher than Yi *et al*. (2021) in prairie grass species (0.045). Usually, genetic differentiation is caused by the lack of effective gene exchange. Centipedegrass has a wide geographical distribution, including Jiangsu, Zhejiang, Fujian, Hunan, Hubei and other regions, which limits the gene exchange between distant germplasm and may lead to a certain degree of genetic differentiation between different geographical populations. Our results also substantiated a significant correlation between geographical and genetic distances (r = 0.385352, p = 0.000140), indicating that geographical isolation led to genetic differentiation, similar to findings reported by Chen *et al*. (2020). The geographical distribution of the 23 accessions collected in this study was relatively dispersed, and the geographical distance was heterogeneous. These factors affected the gene exchange between geographical groups, resulting in greater genetic differences. In addition, climate can lead to genetic variation through natural selection, and environmental adaptability is an important factor in genetic differentiation. Our results showed a significant correlation between BIO14, BIO15, BIO17 and genetic distance, and these three climatic factors are associated with rainfall. This finding may be due to the fact that only some genotypes may survive and prevail with rainfall, which may lead to a decrease in genetic diversity among species populations and even altered gene interactions, consistent with findings reported by Tan *et al*. (2018).

Conclusion

In this study, we evaluated the genetic diversity and population genetic structure of 23 wild centipedegrass accessions by SRAP, PCoA, UPGMA and AMOVA. The UPGMA tree map divided all accessions into two clusters, which was roughly consistent with the results of PCoA. AMOVA revealed that the genetic variation within geographical groups was greater than between geographical groups. Overall, the findings of this study can help better understand the genetic diversity of centipedegrass and lay the groundwork for future research.

Additional files

Table S1. Accession number and collection source of the centipedegrass.

Table S2. Seven morphological traits measured in 23 centipedegrass.

Table S3. Primer name and sequence of SRAP.

Table S4. Q value of 23 cetipedegrass accessions in two groups.

Table S5. Distribution of the Q value of cetipedegrass germplasm in the 3 geographical groups.

Table S6. Correlation data of climate data and geographical groups mental analysis.

Fig. S1. Geographical distribution of 23 wild centipedegrass species used in this study.

Fig. S2. Amplification profiles of 23 cetipedegrass accessions with primer combination M15 + E03 (left), M15 + E08 (right), M9 + E14 (left) and M9 + E20 (right) (accessions 1 to 23 from left to right).

Fig. S3 UPGMA dendrogram of 23 centipedegras accessions based on genetic distance.

Fig. S4. UPGMA dendrogram delineating 23 wild cetipedegrass accessions based on seven morphological traits.

Fig. S5. STRUCTURE estimation of the number of subgroups for the K values ranging from 1 to 10, by delta K (ΔK) values.

Author Contributions

Xiaoyun Wang performed the experiments, analyzed the data, prepared figures and/or tables, and drafted the work or revised it critically for important content.

Wenlong Gou performed the experiments, analyzed the data, and drafted the work or revised it critically for important content

Ting Wang performed the experiments, analyzed the data, and prepared figures and/or tables.

Yanli Xiong analyzed the data, and drafted the work or revised it critically for important content.

Yi Xiong performed the experiments, and prepared figures and/or tables.

Qingqing Yu performed the experiments, and prepared figures and/or tables.

Zhixiao Dong analyzed the data, and prepared figures and/or tables.

Xiao Ma conceived and designed the experiments, and drafted the work or revised it critically for important content.

Nanqing Liu conceived and designed the experiments, and drafted the work or revised it critically for important content.

Junming Zhao conceived and designed the experiments, analyzed the data, and drafted the work or revised it critically for important content.

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References

Budak H, Shearman RC, Parmaksiz I, and Dweikat I. 2004. Comparative analysis of seeded and vegetative biotype buffalograsses based on phylogenetic relationship using ISSRs, SSRs, RAPDs, and SRAPs. *Theoretical and Applied Genetics* **109(2)**:280-288 DOI 10.1007/s00122-004-1630-z.

Cai XY, Fu JY, Li X, Peng LL, Yang LQ, Liang YH, Jiang MY, Ma J, Sun LX, Guo BM, and Yu XF. 2022. Low-molecular-weight organic acid-mediated tolerance and Pb accumulation in centipedegrass under Pb stress. *Ecotoxicology and Environmental Safety* **241**:113755-113755
DOI 10.1016/J.ECOENV.2022.113755.

Chen ZH, Miao JM, Zhong JC, Ma X, Chen SY, and Zhang XQ. 2009. Genetic diversity ofwild Elymus nutans germplasm detected by SRAP markers. *Acta Pratacultural Science* **18**:192-200 DOI CNKI:SUN:CYXB.0.2010-01-025.

Chen G, Bo PT, Yu SH, Lu AJ, Wang QC, Wang JX, and Feng J. 2020. Study on Genetic Diversity of Natural Quercus mongolica Populations in Liaoning Province Revealed by SSR Markers. *Journal of Shenyang Agricultural University* **51(6)**:727-733 DOI 10.3969/j.issn.1000-1700.2020.06.012.

Dent AE, and Bridgett MV. 2012. STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. *Conservation Genetics Resources* **4(2)**:359-361
DOI 10.1007/s12686-011-9548-7.

Forsberg NEG, Russell J, Macaulay M, Leino MW, and Hagenblad J. 2014. Farmers without borders—genetic structuring in century old barley (Hordeum vulgare). *Heredity* **114**:195-206 DOI 10.1038/hdy.2014.83.

Gawali CW, Bhoite KD, Pardeshi SR. 2006. Genetic divergence in sesame (Sesamum indicum L.). *Research on Crops* **7(3)**:758-759

Gao SM, Cong RC, Gao L, Zhu YY, and Zhou Y. 2020. Genetic diversity analysis of phenotypic character and SRAP molecular markers in 45 tree peony cultivars. *Brazilian Journal of Botany*

43:1-12 DOI 10.1007/s40415-020-00596-6.

Hanna WW. 1995. Centipedegrass—Diversity and Vulnerability. *Crop Science* **35(2)**:332-344 DOI 10.2135/cropsci1995.0011183X003500020007x.

Hanna WW, and Burton GW. 1978. Cytology, Reproductive Behavior, and Fertility Characteristics of Centipedegrass1. *Crop Science* **18(5)**:835-837 DOI 10.2135/cropsci1978.0011183X001800050038x.

He QQ, Liu CQ, Li JJ, Wang JJ, Yao X, Zhou SJ, Chen Y, and Wang HR. 2022. Cloning of EoNLA gene in Eremochloa ophiuroides and the transgenic Arabidopsis phenotypic characterization under various phosphorus levels. *Journal of Nanjing Forestry University (Natural Science Edition)* **46(3)**:134-142

Huang LK, Zhang XQ, Xie WG, Zhang J, Cheng L, and Yan HD. 2012. Molecular diversity and population structure of the forage grass Hemarthria compressa (Poaceae) in south China based on SRAP markers. *Genetics and Molecular Research* **11(3)**:2441-50 DOI 10.4238/2012.May.24.3.

Islam MA, and Hirata M. 2010. Centipedegrass (Eremochloa ophiuroides (Munro) Hack.): Growth behavior and multipurpose usages. *Grassland Science* **51(3)**:183-190 DOI 10.1111/j.1744-697X.2005.00014.x.

Li G, and Quiros CF. 2001. Sequence-related amplified polymorphism (SRAP), a new marker system based on a simple PCR reaction: its application to mapping and gene tagging in Brassica. *Theoretical and Applied Genetics* **103(2-3)**:455-461 DOI 10.1007/s001220100570.

Li J, Guo H, Zong J, Chen J, Li D, and Liu J. 2020. Genetic diversity in centipedegrass [Eremochloa ophiuroides (Munro) Hack.]. *Horticulture Research* **7**:4 DOI 10.1038/s41438-019-0228-1.

Li RF, Ding HF, Wang CN, Lu LY, and Zhang XD. 2019. Genetic Diversity of Clover by SRAP. *Agricultural Biotechnology* **8**:10-12 DOI CNKI:SUN:AGBT.0.2019-05-002.

Liu MX, Yang SY, Guo ZF, Lin XP. 2008. Somatic embryogenesis and plant regeneration in centipedegrass (Eremochloa ophiuroides [Munro] Hack.). In Vitro Cellular & Developmental Biology: Plant 44(2):100-104 DOI 10.1007/s11627-008-9115-4.

Liu J, Hanna W, and Elsner E. 2003. Morphological and Seed Set Characteristics of Centipedegrass Accessions Collected in China. *Economic Botany* **57(3)**:380-388 DOI 10.1663/0013-0001(2003)057[0380:MASSCO]2.0.CO;2.

Massa AN, Larson SR, Jensen KB, and Hole DJ. 2001. AFLP Variation in Bromus Section Ceratochloa Germplasm of Patagonia. *Crop Science* **41(5)**:1609-1616 DOI 10.2135/cropsci2001.4151609x.

Milla-Lewis SR, Kimball JA, Zuleta MC, Harris-Shultz KR, Schwartz BM,and Hanna WW. 2012. Use of sequence-related amplified polymorphism (SRAP) markers for comparing levels

of genetic diversity in centipedegrass (Eremochloa ophiuroides (Munro) Hack.) germplasm. *Genetic Resources and Crop Evolution* **59(7)**:1517-1526 DOI 10.1007/s10722-011-9780-8.

Peakall ER. 2012. GenAlEx 6.5: genetic analysis in Excel. Population genetic software for teaching and research—an update. *Bioinformatics* **28**:2537-2539

Pritchard JK, Stephens M, and Donnelly P. 2000. Inference of Population Structure Using Multilocus Genotype Data. *Genetics* **155(2)**:9197-9201
DOI 10.1093/GENETICS/155.2.945.

Riek JD, Calsyn E, Everaeart I, Loose MD, and Bockstaele EV. 2001. Alternatives for the assessment of Distinctness, Uniformity and Stability of sugar beet varieties bases on AFLP data. *Theoretical and Applied Genetics* **103(8)**:1254-1265
DOI 10.1007/s001220100710.

Robarts DWH, and Wolfe AD. 2014. Sequence-Related Amplified Polymorphism (SRAP) Markers: A Potential Resource for Studies in Plant Molecular Biology. *Applications in Plant Sciences* **2(7)**:1400017-1400017
DOI 10.3732/apps.1400017.

Shamustakimova AO, Mavlyutov Y, and Klimenko IA. 2021. Application of SRAP Markers for DNA Identification of Russian Alfalfa Cultivars. *Russian Journal of Genetics* **57(5)**:540-547 DOI 10.1134/S1022795421050112.

Sun M, Dong Z, Yang J, Wu W, and Ma X. 2021. Transcriptomic resources for prairie grass (Bromus catharticus): expressed transcripts, tissue-specific genes, and identification an*d validation of EST-SSR markers. BMC Plant Biology* **21(1)**:264
DOI 10.1186/S12870-021-03037-Y.

Susana R M, Jennifer A K, Zuleta MC, Harris-Shultz KR, Schwartz BM, and Hanna WW. 2012. Use of sequence-related amplified polymorphism (SRAP) markers for comparing levels of genetic diversity in centipedegrass (Eremochloa ophiuroides (Munro) Hack.) germplasm. *Genetic Resources and Crop Evolution* **59(7)**:1517-1526 DOI 10.1007/s10722-011-9780-8.

Tan J, Wan J, Luo F, and Yu FH. 2018. Relationships between Genetic Diversity of Vascular Plant Species and Climate Factors. *Journal of Resources and Ecology* **9(6)**:663-672 DOI 10.5814/j.issn.1674-764x.2018.06.009.

Wu FF, Chen JH, Wang JL, Wang XG, Lu Y, Ning YM, and Li YX. 2019. Intra-population genetic diversity of Buchloe dactyloides (Nutt.) Engelm (buffalograss) determined using morphological traits and sequence-related amplified polymorphism markers. *3 Biotech* **9(3)**:97
DOI 10.1007/s13205-019-1632-9.

Xuan JP, Gao H, and Liu JX. 2005. RAPD analysis of a population of Eremochloa ophiuroides in China. *Acta Pratacul. Sci* **4**:47-52
DOI 10.3321/j.issn:1004-5759.2005.04.008.

Yi LM, Dong ZX, Lei Y, Zhao JM, Xiong YL, Yang J, Xiong Y, Gou WL, and Ma X. 2021. Genetic Diversity and Molecular Characterization of Worldwide Prairie Grass (Bromus

catharticus Vahl) Accessions Using SRAP Markers. *Agronomy* **11(10)**:2054-2054 DOI 10.3390/AGRONOMY11102054.

Yuan X, Tu MY, He YL, Wang W, Li J, and Zhou S. 2018. Analysis of Genetic Diversity in 73 Kentucky Bluegrass Materials by SSR and SRAP Markers. *Notulae Botanicae Horti Agrobotanici Cluj-Napoca* **46(2)**:327 DOI 10.15835/nbha46210916.

Zeller Katherine A, Creech Tyler G, Millette Katie L, Crowhurst Rachel S, Long Robert A, Wagner Helene H, Niko BN, and Landguth Erin L. 2016. Using simulations to evaluate Mantel-based methods for assessing landscape resistance to gene flow. *Ecology and Evolution* **6(12)**:4115-28 DOI 10.1002/ece3.2154.

Zeng B, Zhang XQ, Lan Y, and Yang WY. 2008. Evaluation of genetic diversity and relationshipsin orchardgrass (Dactylis glomerata L.) germplasm based on SRAP markers. *Canadian Journal of Plant Science* **88(1)**:53-60 DOI 10.4141/CJPS07017.

Zhao QL, Bai C, and Liang X. 2011. An analysis by ISSR of genetic diversity in Eremochloa ophiuroides in China. *Chinese Journal of Tropical Crops* **32(1)**:110-115 DOI 10.3969/j.issn.1000-2561.2011.01.023.

Zheng YQ, Xu SJ, Liu J, Zhao Y, and Liu JX. 2017. Genetic diversity and population structure of Chinese natural bermudagrass [Cynodon dactylon (L.) Pers.] germplasm based on SRAP markers. *Plos One* **12(5)**:e177508 DOI 10.1371/journal.pone.0177508.

Figure 1

PCoA and Population structure (K=2) of 23 wild centipedegrass accessions based on SRAPmakers.

SC: from Sichuan province, CQ: from Chongqing municipality, OT: Other accessions except Sichuan and Chongqing.

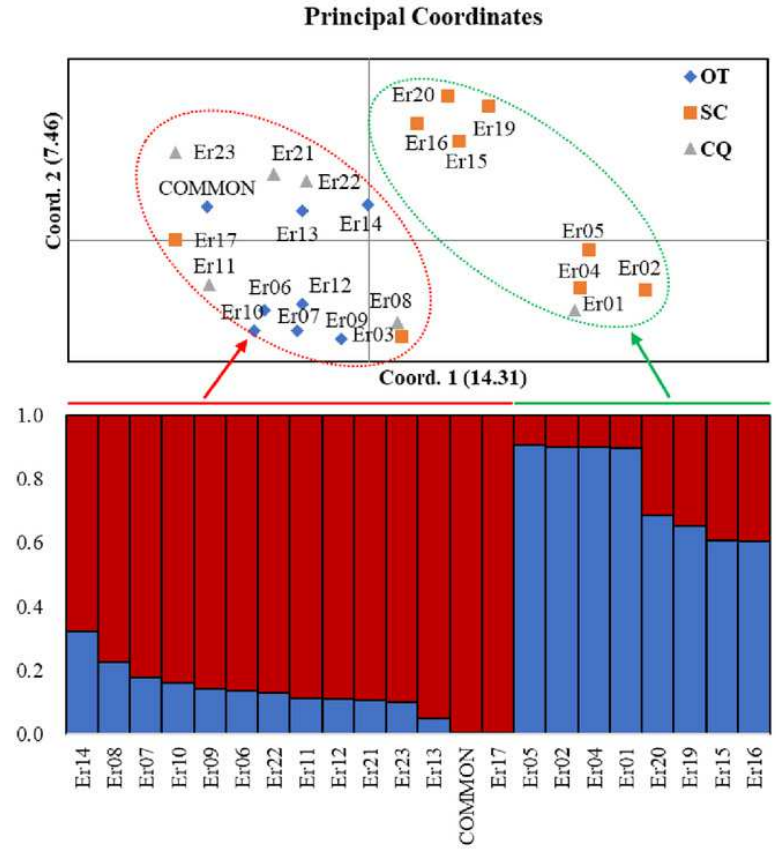

Fig. 1 PCoA and Population structure (K=2) of 23 wild centipedegrass accessions based on SRAP makers. SC: from Sichuan province, CQ: from Chongqing municipality, OT: Other accessions except Sichuan and Chongqing.

Figure 2

Heatmap of correlation between genetic distance and climatic factors.

BIO1: Annual Mean Temperature; BIO2: Mean Diurnal Range; BIO3: Isothermality; BIO4: Temperature Seasonality;BIO5: Max Temperature of Warmest Month; BIO6: Min Temperature of Coldest Month; BIO7: Temperature Annual Range; BIO8: Mean Temperature of Wettest Quarter; BIO9: Mean Temperature of Driest Quarter; BIO10: Mean Temperature of Warmest Quarter; BIO11: MeanTemperature of Coldest Quarter; BIO12: Annual Precipitation; BIO13: Precipitation of WettestMonth; BIO14: Precipitation of Driest Month; BIO15: Precipitation Seasonality; BIO16:Precipitation of Wettest Quarter; BIO17: Precipitation of Driest Quarter; BIO18: Precipitation of Warmest Quarter; BIO19: Precipitation of Coldest Quarter. SC: from Sichuan province, CQ: from Chongqing municipality, OT: Other accessions except Sichuan and Chongqing. **: Very significant difference, *: Significant difference.

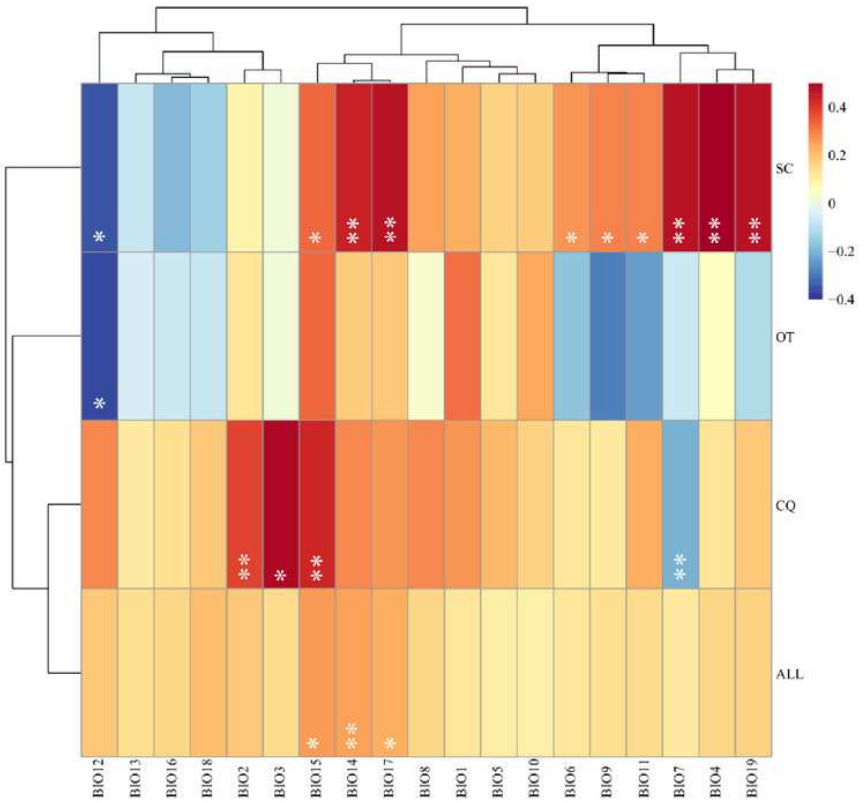

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Table 1(on next page)

Polymorphism of SRAP markers in centipedegrass accessions.

TNB: total of bands; NPB: the number of polymorphic bands; PPB: percentage of polymorphic bands; PIC: polymorphic information content; MI: marker index; RP: resolving power.

1 **Table 1** Polymorphism of SRAP markers in centipedegrass accessions.

Primer Pairs	TNB	NPB	PPB	PIC	RP	MI
M01E03	14	10	71.43	0.248	4.78	1.77
M20E05	22	14	63.64	0.213	6.43	1.90
M01E14	15	11	73.33	0.199	3.83	1.61
M01E20	10	9	90.00	0.263	3.48	2.13
M01E07	23	16	69.57	0.239	7.91	2.66
M01E09	17	13	76.47	0.281	6.87	2.79
M06E03	18	10	55.56	0.196	4.70	1.09
M06E06	17	7	41.18	0.121	2.78	0.35
M06E07	14	11	78.57	0.224	4.43	1.93
M06E09	21	11	52.38	0.170	4.43	0.98
M06E12	13	9	69.23	0.242	4.70	1.51
M06E19	14	6	42.86	0.164	3.04	0.42
M06E04	19	10	52.63	0.215	6.00	1.13
M07E04	20	10	50.00	0.185	5.39	0.93
M07E09	13	6	46.15	0.136	2.35	0.38
M07E07	12	2	16.67	0.114	2.09	0.04
M09E03	17	8	47.06	0.196	4.61	0.74
M09E10	19	10	52.63	0.208	5.65	1.10
M09E14	17	13	76.47	0.229	5.22	2.28
M09E20	19	15	78.95	0.264	6.96	3.13
M10E03	17	11	64.71	0.238	6.43	1.70
M10E06	15	9	60.00	0.186	3.48	1.00
M10E08	19	15	78.95	0.242	6.00	2.87
M10E15	14	9	64.29	0.227	4.43	1.32
M11E01	23	21	80.77	0.270	9.91	4.58
M11E06	20	17	85.00	0.258	6.96	3.73
M11E09	22	17	77.27	0.285	9.39	3.74
M11E10	17	9	52.94	0.196	4.61	0.93
M11E14	17	11	64.71	0.223	5.22	1.59
M12E02	16	12	75.00	0.272	6.09	2.45
M12E06	15	12	80.00	0.270	5.83	2.59
M12E08	18	11	61.11	0.213	5.48	1.43
M12E09	14	9	64.29	0.263	5.30	1.52
M12E03	18	15	83.33	0.252	6.09	3.15
M12E19	19	15	78.95	0.312	9.13	3.69
M14E14	12	8	66.67	0.255	4.26	1.36
M14E04	13	10	76.92	0.270	5.39	2.08
M14E07	7	3	42.85	0.188	2.00	0.24
M15E15	18	13	72.22	0.270	7.13	2.54
M15E03	17	13	76.47	0.278	6.78	2.93
M15E08	19	13	68.42	0.274	7.91	2.44
M16E03	18	10	55.56	0.190	4.43	1.05
M16E08	22	15	68.18	0.233	6.78	2.38

M16E10	14	8	57.14	0.224	4.61	1.02
M17E06	22	11	50.00	0.212	6.87	1.17
M17E08	13	11	84.62	0.264	4.78	2.46
M17E09	14	12	85.71	0.299	6.00	3.07
M17E10	10	9	90.00	0.298	4.09	2.41
M17E12	13	6	46.15	0.149	2.61	0.41
M17E14	17	12	70.59	0.264	6.52	2.24
M17E04	13	8	61.54	0.202	3.57	0.99
M18E06	19	13	68.42	0.257	7.22	2.28
M18E10	22	17	77.27	0.240	6.96	3.15
M19E12	16	8	50.00	0.188	4.00	0.75
M20E06	22	12	54.55	0.168	5.04	1.10
	919	606				
me	16.7	11.01	65.80	0.228	5.4	1.85

TNB: total of bands; NPB: the number of polymorphic bands; P percentage of polymorphic bands; PIC: polymorphic information content; MI: marker index; RP: resolving power.

Table 2(on next page)

Genetic diversity estimation for three geographical groups of centipedegrass accessions.

N: accessions number; Na: the allele number; Ne: effective number of alleles; I: Shannon information index; He: Expected heterozygosity; uHe: Unbiased expected heterozygosity. SC: from Sichuan province, CQ: from Chongqing municipality, OT: Other accessions except Sichuan and Chongqing.

Table 2 Genetic diversity estimation for three geographical groups of centipedegrass accessions.

	N	Na	Ne	I	He	uHe
OT	8.000	1.145	1.220	0.206	0.134	0.143
SC	9.000	1.491	1.326	0.312	0.201	0.213
CQ	6.000	1.255	1.260	0.244	0.158	0.173
Mean	7.667	1.297	1.269	0.254	0.165	0.176

N: accessions number; Na: the allele number; Ne: effective number of alleles; I: Shannon information index; He: Expected heterozygosity; uHe: Unbiased expected heterozygosity. SC: from Sichuan province, CQ: from Chongqing municipality, OT: Other accessions except Sichuan and Chongqing.

Table 3(on next page)

Analysis of molecular variance (AMOVA) based on SRAP markers for geographical groups of centipedegrass accessions.

df: degree of freedom; SS: square deviation; MS: mean square deviation; Est.Var: exist variance; Fst: coefficient of genetic differentiation; PMV: Percentages of molecular variance.

Table 3 Analysis of molecular variance (AMOVA) based on SRAP markers for geographical groups of centipedegrass accessions.

Source	df	SS	MS	Est. Var.	Fst	PMV%
Among geo-groups	2	23.450	11.725	0.770	0.115	12%
Within geo-groups	20	118.028	5.901	5.901		88%
Total	22	141.478		6.671		100%

df: degree of freedom; SS: square deviation; MS: mean square deviation; Est.Var: exist variance; Fst: coefficient of genetic differentiation; PMV: Percentages of molecular variance.

Table 4(on next page)

Pairwise population PhiPT values among three geographical groups of centipedegrass accessions.

SC: from Sichuan province, CQ: from Chongqing municipality, OT: Other accessions except Sichuan and Chongqing.

Table 4 Pairwise population PhiPT values among three geographical groups of centipedegrass accessions.

	OT	CQ	SC
OT	0.000		
CQ	0.092	0.000	
SC	0.180	0.051	0.000

SC: from Sichuan province, CQ: from Chongqing municipality, OT: Other accessions except Sichuan and Chongqing.