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Analysis of SSR Characterization in Transcriptome for Pampass Grass *Cortaderia selloana*

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ABSTRACT

Background: The perennial herbaceous pampas grass (*Cortaderia selloana*: Poaceae) integrates ecological and ornamental functions, serving both environmental rehabilitation and landscape enhancement. Its robust stress tolerance also positions it as a valuable genetic resource for improving resilience in other plant species. The study aimed to analyze characteristics of simple sequence repeats (SSRs) in the transcriptome of pampass grass (*Cortaderia selloana*) to lay a foundation for developing molecular markers for this species.

Methods: Using *C. selloana* leaves, high-throughput sequencing was performed on the NovaSeq × Plus platform. SSR loci were identified from transcriptome data using MISA software, followed by analysis of their distribution and sequence characteristics.

Result: A total of 333.731 reference sequences (unigenes) were obtained, among which 20.684 SSR loci were mined (frequency of occurrence 6.20%, average distribution distance 7.47 kb). SSR repeat motifs were dominated by mononucleotides (7781 SSRs, accounting for 37.62%), trinucleotides (8232 SSRs, 39.80%) and dinucleotides (4064 SSRs, 19.65%). Among 171 repeat motifs, A/T mononucleotide motifs predominated (91.34%), followed by AG/CT dinucleotide motifs (60.58%). SSR repeats were mostly distributed within the 6-15 range, with numbers of all six nucleotide repeat types progressively decreasing as motif repeat count increased. SSR lengths were mostly concentrated in the 12-40 bp range, with SSRs within this length range accounting for 56.90% of all SSR loci. Among these, 3329 SSR loci (16.10%) exhibited higher polymorphism potential (length > 20 bp). SSR loci in *C. selloana* exhibit high frequency, diverse repeat motifs, high polymorphism potential and considerable potential for research on genetic diversity, molecular identification of cultivars and development of novel molecular markers in this species.

Key words: *Cortaderia selloana*, Simple sequence repeat (SSR) loci, Transcriptome.

INTRODUCTION

The perennial herbaceous pampas grass (*Cortaderia selloana*: Poaceae) is widely used in landscape design and the cut flower industry because of its tall, upright growth habit and dense, ornamental panicles. Beyond its aesthetic value, however, *C. selloana* is remarkably stress resistant, and tolerates cold, salinity, drought and waterlogging. Besides, it can survive in both moist and arid soils (Zhang, 2021). Consequently, this species has considerable potential for use in ecological restoration. Because its extensive root system penetrates 30-50 cm into soil, it can be used to stabilize slopes and reduce erosion, making it an ideal species to plant for revegetating embankments and riparian zones. This species also has considerable potential for saline-alkali soil remediation, because it actively secretes organic acids and adsorbs ions through its roots, lowering soil salinity and improving aggregate structure. It has become a pioneer species for vegetation restoration in saline-affected areas of northern and northwestern China. It is also highly adaptable to wetland environments, purifies water by absorbing nutrients (*e.g.*, nitrogen and phosphorus) and plays an important role in rehabilitating degraded aquatic ecosystems. Thus, *C. selloana* integrates ecological and ornamental functions, serving both environmental rehabilitation and landscape enhancement. Its robust stress tolerance also positions it as a valuable genetic resource for improving resilience in other plant species. However, as a cross-pollinating species with a complex genetic background, research on germplasm identification, genetic diversity analysis and molecular-assisted breeding for *C. selloana* is limited.

Traditional morphological markers are more susceptible to environmental influences, whereas simple sequence repeat (SSR) molecular markers have become important in plant genetic research because of their codominant nature, high polymorphism, and technical simplicity (Sun *et al*., 2022; Raghu *et al*., 2019). They are widely applied in the construction of genetic linkage maps, in-depth investigation of germplasm genetic diversity, analysis of genetic relationships and differentiation and population structure studies (Yan *et al*., 2020). Currently, SSR primer development using transcriptome data has been successfully

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implemented for multiple plant species, such as *Vigna radiata* (greengram) (Das *et al*., 2023), *Pisum sativum* (pea) (Singh *et al*., 2022), *Elymus sibiricus* (Siberian wildrye) (Zhang *et al*., 2019), *Dactylis glomerata* (orchardgrass) (Huang *et al*., 2015) and *Melilotus albus* (white sweetclover) (Yan *et al*., 2017).

Given the incomplete genomic annotation of *C. selloana*, mining SSR loci in functional gene regions based on transcriptome sequencing is an efficient approach. This study analyzes the composition and sequence characteristics of SSR loci distributed across the *C. selloana* transcriptome. Objectives are to lay the foundation for germplasm evaluation, genetic map construction and molecular-assisted breeding. These advances will facilitate the effective use of *C. selloana* in ecological restoration applications.

MATERIALS AND METHODS

Plant materials

The experiment was conducted from March 2024 to March 2025 in the greenhouse of the College of Landscape Architecture, Liaoning Agricultural Vocational and Technical College and at Shanghai Majorbio Bio-Pharm Technology Co., Ltd. *Cortaderia selloana* seeds were purchased from Beijing Huayuanli Agricultural Technology Co. Seeds were sown in pots for germination and maintained in a greenhouse under uniform cultivation practices. At the 6-8-leaf stage, the fourth or fifth fully expanded leaves were collected from each seedling, pooled, flash-frozen in liquid nitrogen and stored for subsequent analysis.

Experimental procedures

Transcriptome library construction, sequencing, and assembly

Total RNA was extracted from *C. selloana* leaves using Tiangen RNA extraction kits. RNA concentration and purity were assessed via NanoDrop 2000 spectrophotometry, while integrity was verified by agarose gel electrophoresis. RNA quality numbers were determined using an Agilent 5300 system. After passing quality control, libraries underwent high-throughput sequencing on a NovaSeq × Plus platform (Shanghai Majorbio Bio-Pharm Technology Co., Ltd.). Raw reads were filtered, quality-controlled and de novo assembled to generate unigenes, which served as reference sequences for subsequent analyses.

SSR screening and statistical analysis

SSR loci within unigenes were identified using MISA (http://pgrc.ipk-gatersleben.de/misa/). Minimum repeat thresholds were set as follows: mononucleotides (10 repeats) and di-/tri-/tetra-/penta-/hexanucleotides (6, 5, 5, 5 and 5 repeats, respectively). Compound SSRs were defined as two SSR loci separated by <100 bp. MISA output was collated using Microsoft Excel 2021 for sequence characterization and data visualization.

RESULTS AND DISCUSSION

SSR analysis of the *Cortaderia selloana* transcriptome

High-throughput sequencing generated 333,731 non-redundant unigenes of average length 463.04 bp and total length 154,530,802 bp (Table 1). Screening these unigenes identified 20,684 SSR loci, representing an SSR frequency of 6.20%. Among these, 18,242 sequences contained SSR loci (SSR incidence 5.47%). Additionally, we detected 1197 unigene sequences harboring compound SSR loci (0.36% of total unigenes) and 987 sequences containing ≥ 2 SSR loci (0.30% of total unigenes).

Types of SSR repeat motifs

SSR repeat motifs ranged from mono- to hexanucleotides, with significant variation in abundances. Mononucleotide (7781; 37.62% of all SSRs) and trinucleotide repeats (8232; 39.80%) predominated, followed by dinucleotide repeats (4064; 19.65%). Tetra- to hexanucleotide repeats each accounted for < 2.00%, while compound SSRs constituted 1197 motifs (5.79%). Distribution patterns revealed pentanucleotide repeats to have the longest average distribution distance (1136.26 kb) but the lowest frequency (0.04%). Conversely, trinucleotide repeats had the shortest average distance (18.77 kb) and highest frequency (2.47%) (Table 2).

Base composition and proportion of SSR repeat motifs

A total of 20,684 SSRs comprising 171 repeat motifs were detected in the *C. selloana* transcriptome, with an overall frequency of occurrence of 6.20% (Table 3). Numbers of six-nucleotide repeat types (mono, di, tri, tetra, penta, and hexa) gradually increased, with 2, 4, 10, 31, 41, and 83 types, respectively (frequencies ranging 0.01%-2.13%). However, the total number of SSR loci trended downward.

In terms of base composition, mononucleotide repeats had the fewest types (2), with A/T being the dominant type, forming 7107 loci, accounting for 91.34% of this motif category. Dinucleotide repeats included 4 types, mainly AG/CT, which formed 2462 loci (60.58% of dinucleotide motifs). The CG/CG type was the least frequent, with only 196 loci (4.82%). Trinucleotide repeats comprised 10 types, with CCG/CGG (2488 loci, 30.22%) being the most abundant, followed by AGG/CCT (1443 loci, 17.53%) and AGC/CTG (1082 loci, 13.14%). Least frequent was ACT/

Table 1: Analysis of SSR in transcriptome of Cortaderla Selloana 'Rosea'.

Index	Value	Index 2	Value 2
Total number of sequences examined	333731	Frequeney of SSR occurrence/%	6.20
Average size of examined sequences/bp	463.04	Number of SSR containing sequences	18242
Total number of identified SSRs	20684	Number of sequencescontaining more than 1 SSR	987
Frequency of SSR incidence/%	5.47	Number of SSRs present in compound formation	1197

AGT (87 loci, 1.06%). Tetranucleotide repeats included 31 types, with AAAG/CTTT (42 loci, 12.80%) the dominant type, then AAAC/GTTT (35 loci, 10.67%). Remainding types accounted for <10%, with the least frequent being ACGT/ACGT and ACCC/GGGT (1 locus each, 0.31% each). Pentanucleotide repeats had 41 types, with AGAGG/CCTCT (27 loci, 19.85%) and AAAAAG/CTTTTT (25 loci, 18.38%) being the most numerous. Hexanucleotide repeats had the highest number of types (83), with AAAAAAG/CTTTTT (14 loci, 9.79%) being most abundant (Table 3).

Table 2: SSR repeat types, numbers and distribution characteristics in transcriptome.

Repeat type	Number	Proportion in total SSR/%	Frequency/%	Distance distribution/kb	Average length/bp
Mono-nucleotide repeats	7781	37.62	2.33	19.86	11.64
Di-nucleotide repeats	4064	19.65	1.22	38.02	17.81
Tri-nucleotide repeats	8232	39.80	2.47	18.77	17.12
Tetra-nucleotide repeats	328	1.59	0.10	471.13	22.43
Penta-nucleotide repeats	136	0.66	0.04	1136.26	28.19
Hexa-nucleotide repeats	143	0.69	0.04	1080.63	34.71

Table 3: Repetition and number of different repeat types.

Repeat type	Motif	Number of motif type	Number	Rate	Frequency
Mono-nucleotide	A/T	2	7107	91.34	2.13
	C/G		674	8.66	0.20
Di-nucleotide repeats	AG/CT	4	2462	60.58	0.74
	AC/GT		715	17.59	0.21
	AT/AT		691	17.00	0.21
	CG/CG		196	4.82	0.06
Tri-nucleotide repeats	CCG/CGG	10	2488	30.22	0.75
	AGG/CCT		1443	17.53	0.43
	AGC/CTG		1082	13.14	0.32
	AAG/CTT		994	12.07	0.30
	AAC/GTT		638	7.75	0.19
	ACG/CGT		514	6.24	0.15
	ACC/GGT		511	6.21	0.15
	ATC/ATG		277	3.36	0.08
	AAT/ATT		198	2.41	0.06
	ACT/AGT		87	1.06	0.03
Tetra-nucleotide repeats	AAAG/CTTT	31	42	12.80	0.01
	AAAC/GTTT		35	10.67	0.01
	AAAT/ATTT		24	7.32	0.01
	ACAT/ATGT		24	7.32	0.01
	AAGG/CCTT		22	6.71	0.01
	AGGG/CCCT		21	6.40	0.01
	ATCC/ATGG		17	5.18	0.01
	Other types		143	43.60	0.04
Penta-nucleotide repeats	AGAGG/CCTCT	41	27	19.85	0.01
	AAAAG/CTTTT		25	18.38	0.01
	AAGAG/CTCTT		8	5.88	<0.01
	AGGGG/CCCCT		6	4.41	<0.01
	AAAAC/GTTTT		6	4.41	<0.01
	AAAAT/ATTTT		5	3.68	<0.01
	Other types		59	43.38	0.02
Hexa-nucleotide repeats	AAAAAG/CTTTTT	83	14	9.79	<0.01
	AAGAGG/CCTCTT		8	5.59	<0.01
	ACCAGC/CTGGTG		6	4.20	<0.01
	AGGCGG/CCGCCT		5	3.50	<0.01
	Other types		110	76.92	0.03

Repeat count distribution of SSR motifs

An inverse relationship existed between repeat count and motif abundance across all six nucleotide repeat types (Table 4). Key distribution patterns emerged. Mononucleotides were primarily distributed at 6-15 repeats (92.94% of mono-SSRs). Dinucleotides were dominated by 6-10 repeats (81.23% of di-SSRs). Trinucleotides were concentrated at 1-10 repeats (98.99% of tri-SSRs). Collectively, these dominant repeat ranges accounted for 90.32% of all SSR loci. Tetra-, penta- and hexanucleotides exhibited preferential low-repeat distributions (≥ 6 repeats), with numbers of their main repeat motifs being 324, 109 and 110, respectively (1.57%, 0.53% and 0.53% of all SSRs, respectively).

Evaluation of SSR availability

After filtering out fragments of length <12 bp, the lengths of SSRs with different motifs in *C. selloana* were analyzed (Table 5). The length of dinucleotide SSRs was mainly concentrated at 12-20 bp (66.78% of all dinucleotide SSRs). The length of trinucleotide SSRs was 12-20 bp (75.66% of all trinucleotide SSRs). For tetra-, penta- and hexanucleotide repeats, SSR lengths were 1-20, 21-40 and 21-40 bp, respectively (56.40%, 86.76% and 76.92% of their respective total numbers). Nucleotide lengths of compound SSRs were mainly concentrated at 20-40, 41-60 and 61-80 bp (25.65%, 21.55% and 17.88% of all compound SSRs, respectively).

SSR lengths were mainly concentrated in the range of 12-40 bp, with 11,769 sequences [56.90% of all SSRs (20,684)]. Among these SSR loci, sequences of 12-20 bp length were most numerous (9127, 44.13%), followed by those of 21-40 bp length (2217, 10.72%). Some 1,112 sequences were >40 bp length (5.38% of all SSRs), with the most numerous being the 15-bp trinucleotide repeat (4640 in total, 22.43%), then 18 bp and 12 bp (7.68% and 6.04% of all SSRs, respectively). There were 3329 SSRs of length >20 bp (16.10% of all SSRs). We speculate that these longer sequences may have greater polymorphism potential.

With the rapid advancement of high-throughput sequencing, reference genomes for numerous plant species have been assembled. However, non-model ornamental grasses-particularly those with complex chromosome ploidy and limited molecular biology research-still lack comprehensive genomic resources. Current transcriptome sequencing of *C. selloana* relies on references from related species within the family, resulting in significantly lower data yields and fewer annotated unigenes compared with more commonly studied plants.

We obtained 333,731 *C. selloana* unigenes through transcriptome sequencing. From these, 20,684 SSR loci were identified. With a frequency of occurrence of 5.47%, frequency of appearance of 6.20% and average distribution distance of 7.47 kb, the SSR loci appear to be sparsely distributed in the transcriptome, possibly related to the

Table 4: Repeat times of different SSR motifs.

Repeat	Motif numbers of different repeat times				Total	Primary motify repetition		Proportion in
type	RN (1-5)	RN (6-10)	RN (11-15)	RN (>15)	Number	Number	Proportion/%	total SSR/%
Mono-nucleotide repeats	0	3857	3375	549	7781	7232	92.94	34.96
Di-nucleotide repeats	0	3301	489	274	4064	3301	81.23	15.96
Tri-nucleotide repeats	5136	3013	48	35	8232	8149	98.99	39.40
Tetra-nucleotide repeats	223	101	3	1	328	324	98.78	1.57
Penta-nucleotide repeats	109	24	1	2	136	109	80.15	0.53
Hexa-nucleotide repeats	110	29	3	1	143	110	76.92	0.53

Note: RN (repeat number).

Table 5: Length distribution of SSR different repeat types in *Cortaderia selloana*.

Repeat types	Length/bp	SSR number	Rate/%	Repeat types	Length/bp	SSR number	Rate/%
Di-nucleotide	12~20	2714	66.78	Hexa-nucleotide	21~40	110	76.92
	21~40	493	12.13		41~60	16	11.19
	41~60	117	2.88		>60	4	2.80
	61~80	33	0.81	Compound	20~40	307	25.65
Tri-nucleotide	12~20	6228	75.66		41~60	258	21.55
	21~40	1101	13.37		61~80	214	17.88
	41~60	27	0.33		81~100	146	12.20
	>60	16	0.19		101~120	158	13.20
Tetra-nucleotide	12~20	185	56.40		121~140	72	6.02
	21~40	88	26.83		141~160	21	1.75
	41~60	3	0.91		161~180	9	0.75
Penta-nucleotide	21~40	118	86.76		181~200	9	0.75
	41~60	4	2.94		>200	3	0.25
	>60	2	1.47				

genomic characteristics of gramineous plants. Li *et al*. (2023) used MISA software to search for SSR loci in 130,393 unigene sequences of the *Narenga porphyrocoma* transcriptome; from 14,233 unigenes, 16,372 SSR loci were obtained, with a frequency of appearance of 10.92% Mao *et al*. (2022) searched SSR loci in the 3rd generation full-length transcriptome of *Psammochloa villosa* and found 93,563 SSR repeat sequences distributed in 56,824 unigenes (appearance frequency 50.83%). In the full-length transcriptome of *Littledalea racemosa* with 30,624 unigene sequences, 14,089 SSR repeat sequences were searched using MISA software (frequency of appearance 46.01%) (Fu *et al*., 2025). Yin *et al.* (2024) searched for SSR loci in 214,676 unigene sequences of the *Hordeum brevisubulatum* transcriptome, and detected 24,877 SSRs in 21,618 unigene sequences (frequency of appearance 11.59%). Wang *et al*. (2022) performed transcriptome sequencing on an *Agropyron mongolicum* hybrid and from 110,115 unigenes obtained 5620 SSR loci after locus searching (frequency of appearance 5.10%) (Wang *et al*., 2022). Compared with these gramineous plants, the SSR frequency of appearance in *C. selloana* is higher than for *A. mongolicum* (5.10%), but lower than for *P. villosa*, *L. racemosa*, *N. porphyrocoma* and *H. brevisubulatum*. The frequency of occurrence of SSR loci in *C. selloana* differs from transcri-ptomes of other gramineous plants. Inherent differences in gene structure of species may explain these differences, although factors such as the size of the analysis database, SSR retrieval tools, and parameter settings of retrieval conditions cannot be excluded (Fu *et al*., 2021).

Regarding repeat types, *C. selloana* SSRs were dominated by mononucleotide (37.62%) and trinucleotide repeats (39.80%), with dinucleotides secondary (19.65%). Tetra- to hexanucleotide repeats collectively comprised <3% of all SSRs. This distribution aligns with patterns in other Poaceae species *L. racemosa* (mono, 36.88%; tri, 39.34%; di, 21.13%) and *H. brevisubulatum* (mono, 50.90%; tri, 28.78%; di, 17.48%) (Yin *et al*., 2021) confirming the prevalence of low-order repeats in grasses. Notably, *C. selloana* exhibited a significantly higher proportion of trinucleotide repeats (39.80%) than *H. brevisubulatum* (28.78%), suggesting enhanced variation potential in coding regions and greater utility for polymorphic marker development.

In terms of motif composition, the dominant motif types in *C. selloana* are consistent with those of Gramineae. Among the 171 repeat motif types detected in the *C. selloana* transcriptome, the mononucleotide A/T type accounts for the highest proportion (91.34%); the dinucleotide is dominated by AG/CT (60.58%) and the trinucleotide CCG/CGG is also high-frequency type (30.22%). This base preference is related to the codon usage preference of Gramineae plants and the high mutation rate in AT-enriched regions of the transcriptome. The conclusion that the dominant motif in dinucleotide repeats of SSR loci is AG/CT and the dominant motif in trinucleotide repeats of *C. selloana* (CCG/CGG) is consistent with results for other Gramineae plants such as *Teosinte* (Li *et al*., 2023) and *Coix lacryma-jobi* (Ouyang *et al*., 2021). This observation also supports research reporting the dominant repeat motifs of dinucleotides and trinucleotides in SSRs of most monocotyledonous plants to be AG/CT and CCG/CGG, respectively (Kantety *et al*., 2002). However, dominant motifs in plants such as *Diospyros rhombifolia* (Wang *et al*., 2022) and *Brassica campestris chinensis* var. *purpuraria* differ (Xi *et al*., 2022), possibly because of species specificity or search conditions. The repeat motif types of nucleotides of different species vary, and the dominant motifs in different repeat types also change. This is conducive to development of SSR molecular markers. Among them, the CCG/CGG motif also has the highest proportion in *L. racemosa* (31.32%) and *N. porphyrocoma* (20.26%), indicating its evolutionary conservatism in monocotyledons.

High polymorphism-the core value of SSR markers-depends on repeat unit counts and the abundance of ≥20 bp fragments. Generally, SSRs ≥20 bp exhibit high polymorphism, and those of 12-20 bp show moderate polymorphism. In *C. selloana*, SSR repeat counts predominantly ranged 6-15 (92.94% of total loci), with lengths concentrated in 12-40 bp (56.90%). Notably, 3329 SSR loci (16.10%) exceeded 20 bp. These longer repeats have enhanced polymorphism potential because of extended motif repetitions, making them promising targets for developing highly informative molecular markers.

Study limitations must be acknowledged. First, the SSR frequency in *C. selloana* (6.20%) is significantly lower than that of *P. villosa* (50.83%) and *L. racemosa* (46.01%), possibly because of sequencing depth or unigene assembly completeness. Second, tetra- to hexanucleotide repeats constituted < 3% of all SSRs, constraining development of complex polymorphic markers. Future efforts could integrate genomic sequencing data to mine genomic SSRs for enhanced marker density and validate high-polymorphism loci through primer screening. These advances will support cultivar identification, genetic linkage map construction and stress-resistance gene localization in *C. selloana*.

CONCLUSION

This study provides the first systematic characterization of SSR distribution patterns in the transcriptome of *C. selloana*, yielding three principal findings. The 20,684 identified SSR loci (frequency 6.20%) were dominated by mononucleotide (37.62%) and trinucleotide repeats (39.80%). Dominant motifs (A/T (mono-), AG/CT (di-) and CCG/CGG (tri-) align with Poaceae evolutionary traits. High polymorphism potential for marker development exists, with 3329 SSR loci (16.10%) exceeding 20 bp length.

The widespread distribution and rich diversity of SSR loci in *C. selloana* represent a robust foundation for efficient marker development. Significant potential exists to apply these results in research on genetic diversity, molecular identification of cultivars and development of novel molecular markers in this species.

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Disclaimers

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Informed consent

The collection of plant seeds conforms to China's regulatory standards and current laws.

Conflict of interest

The authors declare that there are no conflicts of interest regarding the publication of this article. No funding or sponsorship influenced the design of the study, data collection, analysis, decision to publish, or preparation of the manuscript.

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