Molecular Diversity in Sewan Grass (*Lasiurus sindicus* Henr.): A Natural Inhabitant of Hot Arid Ecosystem of Thar Desert

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The 23rd International Grassland Congress (Sustainable use of Grassland Resources for Forage Production, Biodiversity and Environmental Protection) took place in New Delhi, India from November 20 through November 24, 2015.

Proceedings Editors: M. M. Roy, D. R. Malaviya, V. K. Yadav, Tejveer Singh, R. P. Sah, D. Vijay, and A. Radhakrishna

Published by Range Management Society of India

Paper ID: 957
Theme 4. Biodiversity, conservation and genetic improvement of range and forage species
Sub-theme 4.1. Plant genetic resources and crop improvement

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Keywords: Arid ecosystem, Desert grass, Genetic diversity, *Lasiurus sindicus*, Sewan grass

Introduction

Lasiurus sindicus Henr., locally known as "Sewan", a member of family poaceae, is a tufted perennial, forming a more or less oblique and woody rhizomatous rootstock with many shoots arising from the base, often appearing almost bushy. This grass has developed a number of morphological, anatomical and biochemical strategies to withstand the extreme climatic conditions. The leaves show characteristic C_4 NADP-ME type of anatomy and have developed sclerenchyma to impart mechanical strength during drought and high wind. Sewan is a dominating grass species of *Dichanthium-Cenchrus–Lasiurus* type grass lands of hot arid ecosystem of Great Indian Desert, covering western Rajasthan and parts of Pakistan. It grows naturally in wide range of dry areas covering North Africa, Sudano-Sahelian Africa, East Africa and Asia. It thrives well in dry climate receiving annual rainfall below 250 mm prevailing between 25-27°N latitude on well aerated alluvial soils or light sandy soils with a pH of 8.5, rocky ground and gravelly soils. Though this grass tolerates prolonged droughts but has not been found growing in higher rainfall zones and faces a serious threat of becoming an endangered due to changes in the land use pattern, increase in soil moisture regime and overgrazing.

The Sewan grass, considered as the "King of Desert Grasses", is quite palatable and nutritious for the livestock. Crude protein in young leaves varies from 7 to 14% and remains high even at maturity leading to its better suitability for efficient utilization in the animal based agri-horti-pastoral production system prevalent in hyper arid regions of western Rajasthan. In the three districts of western Rajasthan *viz*. Bikaner, Barmer and Jaisalmer the sustainability and productivity of livestock mainly depends on the sewan based pasture system. The present study was undertaken to analyze the extent of genetic variability existing among the *L. sindicus* germplasm, collected from Bikaner, Barmer and Jaisalmer, the diversity rich districts of hyper-arid Rajasthan, using ISSR and RAPD markers, for its importance in determining survival under changing climate.

Materials and Methods

Twenty seven genotypes of *L. sindicus* Henr. were collected from sewan growing area of western arid Rajasthan (Figure 1) covering three districts, Jaisalmer (10 accessions), Barmer (9 accessions) and Bikaner (7 accessions) and 1 (old collection maintained at CAZRI, Jodhpur). All these genotypes are being maintained under field conditions at the Central Research Farm of CAZRI, Jodhpur (India).

Genomic DNA extracted from approx. 1 g of tender leaves using CTAB method was treated with RNase, assessed on 0.8% agarose gel and quantified using UV/VIS spectrophotometer. The quantified DNA was diluted to 25ng/µl concentration for PCR amplification. The PCR reaction carried out in a volume of 25µl consisted of 1x assay buffer, one unit *Taq* DNA polymerase, 200 µM of each dNTPs, 10 pM primers and 50 ng template DNA. PCR amplifications were performed in a CGI-96 thermal cycler with following cycling conditions - initial denaturing at 94°C for 6 min; 44 cycles x [94°C for 1 min, 37°C (RAPD) / 42° C (ISSR) for 1 min, 72°C for 2 min] followed by final extension at 72°C for 7 min. The PCR product was electrophoretically separated on 1.2% agarose gel in 0.5x TBE buffer, containing ethidium bromide (10 mM). GeneRuler™ DNA ladder mix was used as size marker.

Data analysis was performed using NTSYS-pc while, SIMQUAL program was used to calculate the Jaccard's similarity coefficient for pair-wise comparisons based on the proportion of shared fragments produced by the primers. The dendrogram was generated from similarity matrix data by cluster analysis using unweighted pair group method for arithmetic mean (UPGMA). Pairwise population comparisons were performed with an analysis of molecular variance (AMOVA) using Genalex 6.5. Nei's measurements of genetic diversity among natural populations were also calculated, using POPGENE version 1.32 assuming all loci to be dominant and in Hardy–Weinberg equilibrium.

Results and Discussion

A total of 18 RAPD and 14 ISSR markers were screened of which 12 RAPD and 10 ISSR primers amplified distinct and scorable fragments. The comparative analysis of data shows that RAPD markers were more efficient than ISSR with regards to polymorphism detection, as they detected 90% polymorphism in comparison to 74% for ISSR markers. The values of average number of polymorphic bands per assay, polymorphism information content (PIC) and discriminatory power (Dj) were more for RAPD (5.83, 0.222 and 0.78 respectively) than for ISSR (5.7, 0.138 and 0.605 respectively). Though none of the RAPD primers produced unique patterns for all the 27 genotypes, however they could resolve all the genotypes collectively with an average diversity of 42%. Unlike RAPD markers, ISSRs could reveal less diversity (29 %) in the populations Barmer 43 and 44 (84.8 % similarity) remained the most related genotypes based on collective analysis of the two marker systems, however the most distant genotypes were LSGP 2365 and Barmer 47 (50 %). RAPD based AMOVA revealed that genotypes belonging to regions of Jaisalmer and Barmer had more within region similarity, 62 and 65% respectively, compared to Bikaner (43 %). ISSR markers also confirmed higher similarity within regions for Jaisalmer and Barmer collections (75 %). Jointly two markers systems retained the similar trend revealing highest similarity in Barmer (71%) and least in Bikaner (66 %) populations (Figure 2). Polpulations from Jaisalmer and Barmer were most similar while Bikaner remained most distant.

RAPD markers have been efficient over ISSR markers in resolving polymorphism leading to higher PIC and discriminatory power. Better efficiency of RAPD markers was also evident by better clustering at a lower level of similarity. Similarly Cuesta *et al*. (2010) also reported that only RAPD markers from among RAPD, ISSR, AFLP an SAMPL were effective in detecting interclonal variation in micropropagated plants of *Pinus pinea* L. However, ISSR markers have been reported to be more efficient in *Cyamopsis tetragonoloba* (L.) Taub (Sharma *et al.* 2014). The differential resolution of RAPD and ISSR markers may be due to different target portions they bind to in the genome. The greater ability of RAPD over ISSRs may be attributable to their arbitrary nature virtually exploring wider genomic region.

Shannon index for diversity was higher for Bikaner region (0.3570) followed by Jaisalmer (0.3304) and Barmer (0.3047) this is in agreement with average similarities calculated on the basis of Jaccard's similarity matrix, 0.65, 0.70 and 0.71 respectively. On the basis of RAPD marker polymorphism, diversity among Barmer-Jaisalmer region was less (40%), compared to Bikaner-Jaisalmer (46%) and Bikaner-Barmer (46%). Similar trend was revealed by ISSR markers, however, with lower levels of diversity viz. 25, 27 and 30% respectively for Barmer-Jaisalmer, Bikaner-Barmer and Bikaner-Jaisalmer. The higher diversity for allele content and its frequency in Bikaner collections could be because of less harsh conditions and availability of irrigation facilities whereas adaptive pressures might be limiting diversity in harsher climate of Jaisalmer and Barmer region. More similarity and close relationship between collections from Barmer and Jaisalmer can be further explained based on the fact that the collection sites from Barmer and Jaisalmer have similar climatic conditions and occur in continuity covering broader region while collection sites from Bikaner were geographically separated and more localized.

Both AMOVA and Nei's gene diversity index detected considerable genetic diversity among the populations, however, diversity within population was higher. Both RAPD and ISSR markers independently and collectively detected diversity among populations in same range viz. 14, 17 and 15% respectively. The Nei's genetic diversity based on combined RAPD-ISSR analysis, among population was also in the same range (18%). Higher level of within population diversity is important for the survival of *L. sindicus* under unpredictable fragile ecosystem and diversity among populations might have been important in niche specific adaptations.

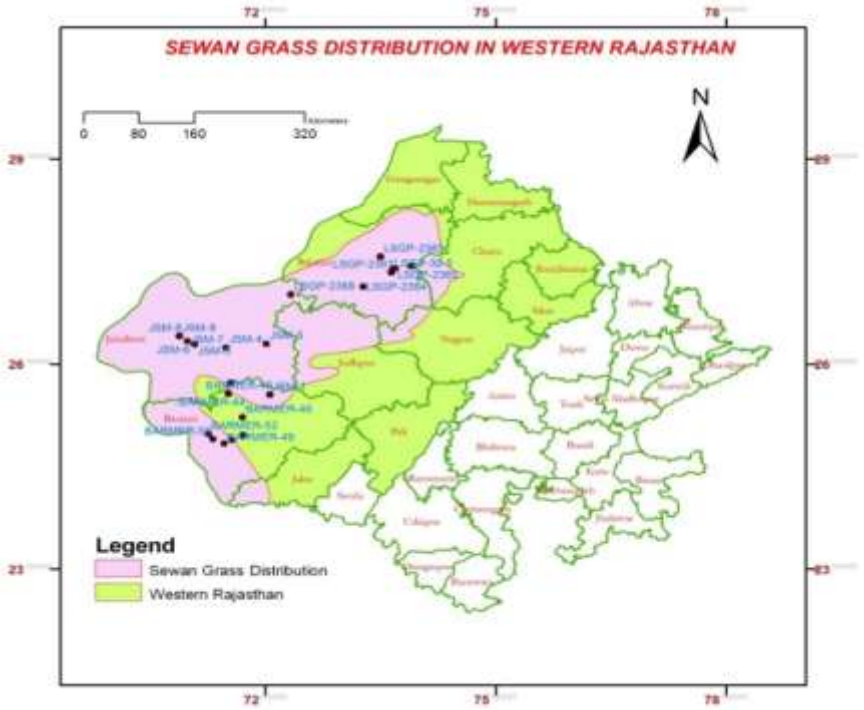

Fig 1: Distribution of sewan grass in Rajasthan with sites of sample collection

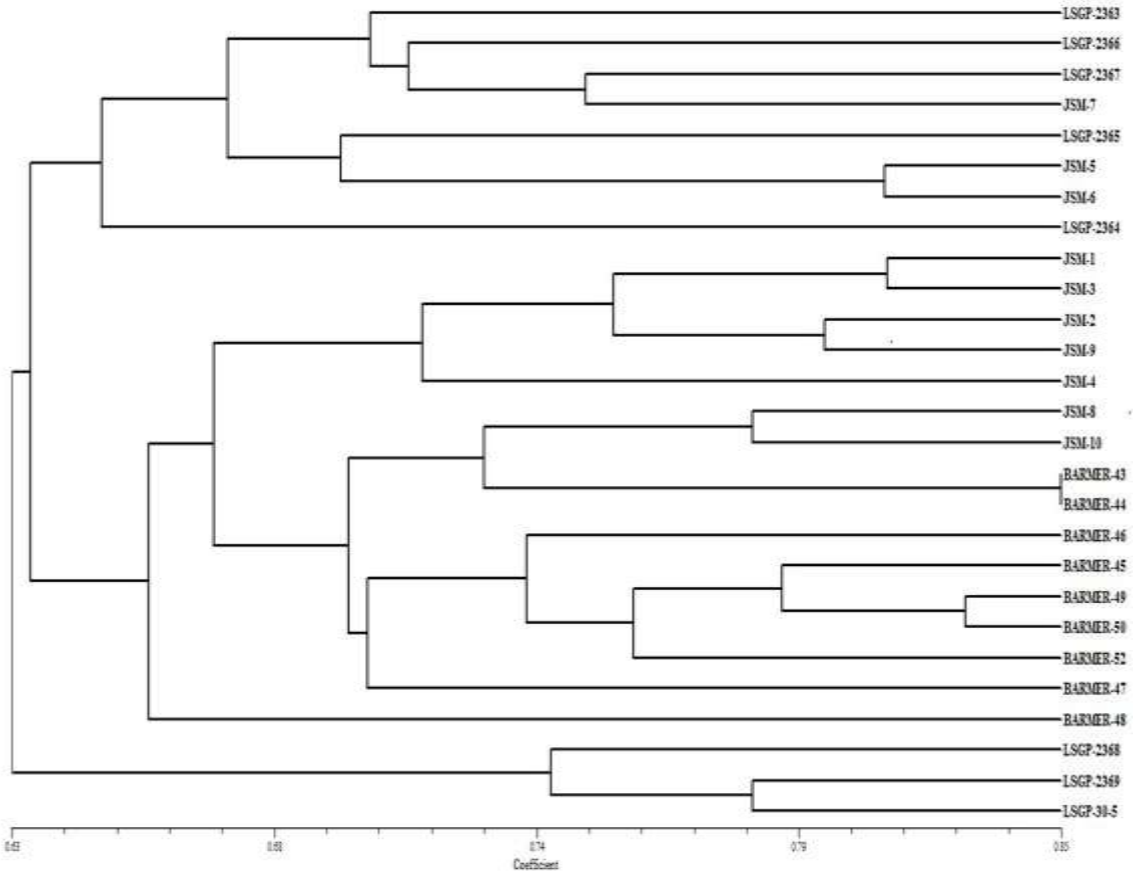

Fig 2: Dendrogram of 27 accessions of *Lasiurus sindicus* obtained using combined data of RAPD and ISSR markers.

Conclusion

The results on the genetic diversity between accessions of *L. sindicus* collected from different regions of the Rajasthan, India obtained in present study will be important to increase our knowledge about the genetic variations and relationship existing between the genotypes and will be important for efficient utilization and conservation, *ex situ* as well as *in situ*, of this important plant species.

References

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