



# Development and Utilization of DNA Markers to Assess Genetic Diversity of Proso Millet (*Panicum miliaceum*) Germplasm

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## Abstract

Proso millet (*Panicum miliaceum*) is one of the oldest cereals with high WUE and short growing-season. It is highly adapted to dry and drought-prone area. It is currently grown USA, Asia (China, Russia, Ukraine, Korea, and India) and Africa. Though there is great advancement in genomics, however, genomic resources in proso millet are very limited. The efficient and cheapest way to develop large numbers of DNA markers and other genomic tools in proso millet is using genomic resources in related grasses. The objectives of the present report are (1) to develop SSR markers using cross-species genomic resources, (2) to determine the genetic diversity in proso millet germplasm. A total of 624 SSR markers (548 from switchgrass, 25 from proso BAC library, 21 from rice, 15 from wheat, 9 from oat and 1 from barley) were used. Total of 66 proso germplasm from 28 countries were used for genetic diversity analysis. Only 8 lines were initially tested to identify polymorphic markers. Cluster analysis (based on UPGMA) was done using NTSYS-pc. Out of 624 SSR primers tested, 464 amplified SSR markers in proso millet. A majority (~62%) of these 464 markers were from switchgrass. Of these 464 markers 283 were polymorphic among 8 lines and 120 of these 283 polymorphic markers were used in 66 germplasm. The 120 SSR markers amplified 730 different alleles. The DNA marker based genetic analysis grouped 66 germplasm into different clusters. Switchgrass genomic information seems to be the most useful for developing DNA markers in proso millet. Markers developed in this study will be helpful for linkage map construction, mapping agronomic traits and future molecular breeding efforts in proso millet.

## Introduction

Proso millet (*Panicum miliaceum* L.) is a small grain cereal with high WUE, short growing season. It is more capable of producing harvestable grain under limited water conditions (drought) and on marginal soils than other cereals.

In USA it is used as bird seed however, it is used as human food in other countries.

It is currently grown USA, Asia (China, Russia, Ukraine, Korea, and India) and Africa. In USA ~90% of proso millet is grown in Colorado, Nebraska, and South Dakota. It contributes annually \$50 million to the bird seed industry.

Current proso millet varieties have very narrow genetic base; therefore, is vulnerable to unforeseen production risks. Genomics, genetics and breeding research in proso millet is extremely limited. Cross-species genomic information is highly useful and cheapest way to develop new markers in orphan crop like proso millet. Genetically diverse germplasm is the base of any breeding program. Therefore, genetic diversity analysis of germplasm is very important for variety improvement.



## Allele distribution

We have scored allele numbers for all the polymorphic markers and it is shown in the Figure 1. The high frequency of 2 and 4 alleles suggests that part of the proso millet genome is tetraploid nature.

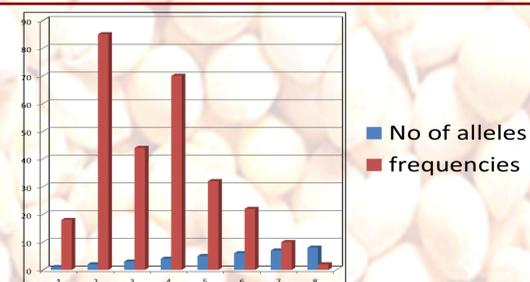


Fig 1. Distribution of SSR alleles based on proso millet 8 lines

## Genetic diversity

The marker analysis illustrated high level of diversity among the germplasm. The genotypes were clustered into three groups:

- Group 1 consisted of 5 lines (3 from USA, 2 from other countries)
- Group 2 consisted of 60 lines. This major group consisted of 4 sub-groups - 2a, 2b, 2c, and 2d
- Group 3 consisted of only one line from China

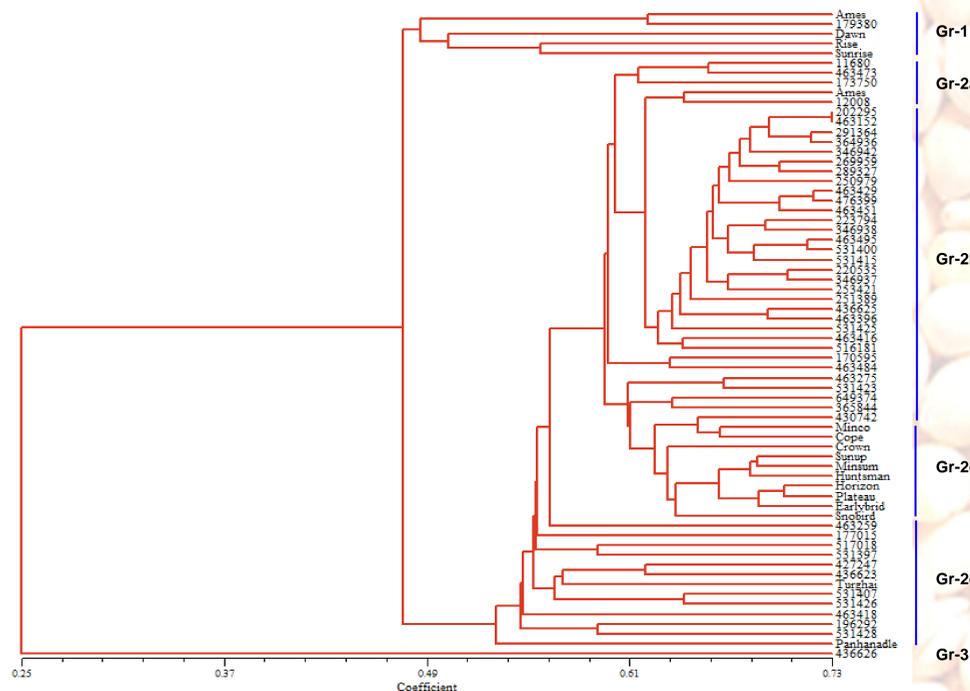


Fig 3. UPGMA dendrogram showing the genetic relationships of 66 proso millet genotypes.

## Objectives

- To develop SSR markers for proso millet using cross-species genomic resources
- To determine the genetic diversity in proso millet germplasm using DNA markers

## Material and Methods

### Plant material

We used 66 highly diversified proso genotypes from 28 countries.

### SSR primer selection, evaluation and screening

We screened total 624 SSR primers. We randomly selected 548 (540 genomic & 8 EST) evenly distributed di, tri, tetra and other composite repeat SSR markers from switchgrass (Liu et al., 2012; Wang et al., 2010); 46 markers from wheat, rice, barley, and oat (Hu et al., 2009); 25 markers from proso millet (Cho et al., 2010). For SSR marker development we screened all 624 SSR primers in panel of 8 proso lines. For genetic diversity study we used total 120 polymorphic SSRs in 66 lines.

### Genetic diversity analysis

Genetic relationship was estimated using NTSYSpc (Numerical Taxonomy Multivariate Analysis System) version 2.2 software. All the 66 genotypes were used. Binary data matrix was generated by scoring each SSR band as present (1) and absent (0). Genetic relationship among the 66 genotypes was assessed by calculating the genetic distance and UPGMA (Unweighted Pair group method with Arithmetic average) for constructing dendrogram.

## Results and Discussion

Out of 624 SSR primers screened, 464 amplified of which 283 were polymorphic. Most of the polymorphic primers have produced 2 or 4 alleles and remaining ranged from 1 to 8 alleles. The di-nucleotide repeats were found at highest frequency in the proso genome. Among the di-nucleotide repeats AG/GA were predominant (43%) followed by AC/CA (21%). Out of 13 tri-nucleotide repeats markers, 7 were polymorphic and 6 were monomorphic. Overall cross species transfer rate from related grass species to proso millet was 74%.

Details	Nos.	Percentage (%)
Primers used	624	N/A
Amplified	464	74
Polymorphic	283	45
Monomorphic	181	29
No amplification	160	26

Table 1: summarizing the number of markers used and the relative percentage

## Conclusions

- High percentage of cross-species SSR markers (74 %) suggests that proso millet genome is very similar to other grasses
- There is 62% cross species transferability between proso millet & switchgrass
- Mostly AG/GA repeats were amplified followed by AC/CA repeat motifs
- New SSRs were able to differentiate 66 proso genotypes into different groups

## References

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