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Proso Millet Breeding Progress, Status of Genomic Resources and Future Aspects of Improvement

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ABSTRACT: Small millets are potentially high nutrient rich crops with great value to human consumption and animal feed. Small millets include small seeded crops of Poaceae family like foxtail millet, finger millet, kodo millet, proso millet, barnyard millet and little millet. Proso millet is low-input and less demanding small millet known for its rich magnesium content. Developing high yielding and nutrient packed proso millet varieties improves industrial adoption and cultivation. Information on genomic resources available in proso millet can improve breeding activities and quicken the genetic gain. Therefore, pre-breeding in proso millet, germplasm and molecular breeding avenues are focus in the article.

Keywords: Genomic resources, pre-breeding, genetic gain, proso millet.

INTRODUCTION

Small millets are grown over wide agro-ecologies, being included in traditional cropping systems. All millets including small millets are ethnic foods in many socio-geographical cultures increasing food diversity. They are major source of sustenance in dry land areas as well as to tribes of hills and plains meeting the food security of region's households. The small millets are highly resilient in adaption. They are nutrient packed whole grain foods high in fibre, quality protein and several micronutrients (Srujana *et al*., 2022).

Proso millet (*Panicum miliaceum* L., 2n=4x=36), commonly known as hog millet or broom corn millet, was initially domesticated in central Asia and Europe (Hunt *et al.,* 2011). Cultivation of proso millet is distributed across India, China, Russia, Japan, Mongolia, USA, Iran, Afghanistan, Iraq, and other East Asian countries. This millet is also called as cheena, variga, panivagaru and bagaru as vernacular names in Indian languages. Proso millet is rich in protein (>10%) with higher amount of essential amino acids, minerals and vitamins (Gomesh *et al.,* 2017). Proso millet is majorly utilised as bird feed and cattle feed.

PREBREEDING, GERMPLASM SCREENING AND SELECTION

The potential health benefit qualities of proso millet besides gluten free and mild flavour are increasing the preference for human consumption and food industries (Wang *et al*., 2016). The morphology and shape of panicle subdivides cultivated type races that include *miliaceum, patentissimum, contractum, compactum* and *ovatum* (Lu *et al*., 2009). Considering the demand, grain yield is the major breeding objective in proso millet improvement programs in addition to heat and drought stress. Landraces haven been primary source of varieties in proso millet and a few more resulted from hybridization efforts. Studies reporting diversity assessment using agro-morphological traits and identified germplasm for breeding programs are limited for proso millet (Vetriventhan and Upadhyaya 2018; Vetriventhan *et al*., 2019; Calamai *et al*., 2020). Humans currently have access to over 29,300 conserved germplasm accessions of proso millet all over the world (Table 1). Europe followed by Asia consist the highest number of accessions being 15,812 and 12,110, respectively. The genebank in ICRISAT, India holds nearly 850 accessions. These accessions were evaluated and a core collection was generated comprising of 106 accessions representing all the five races (Upadhyaya *et al*., 2011). Evaluation of several proso millet germplasm collections consisting of landraces, wild types, varieties and advanced breeding lines provides diversity scenario and line based trait profile for further utilisation in breeding programs. Vetriventhan and Upadhyaya (2018) evaluated 200 accessions of proso millet including the 106 representative core of ICRISAT, India for diversity in morpho-agronomic and nutritional traits. The study identified potential nutrient rich lines with high yielding traits. However, the lines are yet to be tested for regional adaptation that would further enhance their breeding utilization purposes. Proso millet is well adapted to drought areas where the water availability can be as low as 20-50 cm. Habiyaremye *et al.* (2017) identified GR665 and Minsum varieties as good performers in non-irrigated areas. Primary evaluations of 360 proso millet accessions reported high variability, high genetic advance and high heritability for yield contributing traits studied. The use of molecular markers give molecular fingerprint of the accessions and collections in crop improvement. Proso millet accessions have be evaluated for genetic diversity using molecular markers such as AFLP, RAPD, ISSR and SSRs (Karam *et al.,* 2004; Trivedi *et al.,* 2015; Rajput and Santra 2016). SSR markers were derived from sequences of related plant species and utilised to study diversity and genetic similarity coefficient. These SSR were able group the accessions into clusters that corresponded to ecological areas of collection (Hu *et al*., 2009; Rajput *et al*., 2014). SSR based molecular studies aiming for line based DNA profiling identified numerous alleles across accessions and relative geographic structuring (Cho *et al*., 2010; Hunt *et al*., 2011; Rajput and Santra 2016). Diversity analysis using markers derived from coding genomic regions such as intron splice junctions, clustered landraces and varieties differently besides clustering relative to geographic locations (Weining and Henry 1995; Hu *et al*., 2008). Similar study based on 5S ribosomal sequences constructed phylogenic relationships that clustered accessions from China and Russia together and Korean accessions separately (Pak *et al*., 2012). Information generated from these studies are of paramount importance for utilization in breeding programs and conservation purposes. The development of hybrids in future could be initiated from the groups generated using molecular markers in proso millet where the combining ability studies are not reported so far.

VARIETIES, MATING AND METHODS

In India, there are currently 24 varieties of proso millet (Vetriventhan *et al*., 2020). Proso millet is not easily amenable to crossing and hybridisation due to smaller size of inflorescence and high rate of self pollination. Varieties released in India through hybridization are 25% of all proso millet varieties (Table 2). The important varieties are Co(PV), TNAU 151, TNAU 164 and TNAU 202. Majority of the varieties have been developed through selection from landraces.

MOLECULAR BREEDING

Advancements in genomic resources in crops have contributed immensely in several crops in i) developing suitable robust molecular markers capable of providing genomic profile of species, ii) developing better screening methods to varietal identification and trait dissection, iii) understanding biological development mechanism, iv) developing innovative breeder friendly approaches in crop improvement. Over the past years, success in molecular studies using genomic resources resulted in more than 130 cultivars available to farmers (Ellur *et al.,* 2016; Arelli *et al.,* 2017; Varshney *et al*., 2014; Hossain *et al.,* 2022).

Markers developed. In case of proso millet, the genome have been sequenced and made available along with complete sequenced of chloroplast genome sequence. The draft genome size of proso millet is 923 Mb as sequenced from landrace accession 00000390 (Kubesova *et al*., 2010). The number of genes predicted are 55,930 (Zou *et al*., 2019). The sequence needs resequencing with new annotations avoid the present genome gaps. In addition, several reports are available with whole genome sequence data performed for SNP associations**.** The availability of genome sequences enhanced the development of molecular markers and functional markers such as simple sequence repeats(SSR) from related genome or own sequence, single nucleotide polymorphism (SNP), expressed sequence tag(EST) derived markers from transcribed regions besides understanding the diversity of proso millet (Hu *et al*., 2009; Cho *et al.,* 2010; Yue *et al*., 2016; Saha *et al*., 2016). The markers have been used to study genetic relationships among collections and characterization of germplasm and species identification. Besides SSRs, genetic linkage maps, sequence of waxy gene and certain miRNAs are available (Wu *et al*., 2012).

Genetic diversity studies using SSR markers were initially conducted using markers from related species such as rice, barley, oat and switchgrass (Hu *et al.,* 2009; Rajput *et al.,* 2014). Proso millet genome derived SSRs were fist developed by Cho *et al.* (2010) which were further validated and used for diversity studies by Hunt *et al.* (2011). The studies were able to predict the domestication and spread of proso millet from the collections used. Using the species specific and related genomic SSRs, core sets were developed of the collections of USDA-ARS and ICRISAT (Upadhyaya *et al*., 2011; Rajput and Santra 2016). Gene based markers were used in proso millet to understand the mutations and gene based diversity. *Waxy* gene of proso millet was sequence by Hunt *et al.* (2010). The identified mutant alleles were studied in Eurasian and Japanese landraces to identify diversity for *Waxy* gene and identified accessions with high and low waxyness (Hunt *et al*., 2012; Araki *et al*., 2012). An extensive genome wide analysis of population structure of 190 proso millet accessions identified 1882 SNPs that identified eight putative sub populations (Johnson *et al*., 2019). The finding agree from diversity and trait heritability values with validations on public database of phenotypes.

Genes mapped and QTLs identified. The construction of first robust genetic linkage map using SNPs in proso millet have helped in identifying 18 QTLs for agro-morphological traits on 14 linkage groups explaining a phenotypic variance of range 13 to 34 % (Rajput *et al*., 2016). A single report of using genomics identified 10 marker trait associations of several key agronomic traits like seed perimeter, seed length, seed width and seed circularity in 88 accessions of proso millet using 2412 SNPs. The accessions were highly diverse with significant level of admixture. The independent validations of these marker trait associations can be useful in identifying linked markers for utilisation in marker assisted selection.

TANSGENIC RESEARCH AND MOLECULAR MECHANISM

ESTs were used in proso millet to mine miRNAs due to limited genomic resources where, 43 miRNAs were identified and 12 were validated (Wu *et al.,* 2012). The identified miRNAs had gene targets involved in development, stress response and metabolism.

Transcriptome sequencing of drought tolerant and sensitive cultivars in proso millet identified 833 and 2166 differentially expressed genes, respectively. The study with induced water stress identified differential responses due to ROS scavenging system and jasmonic acid signal transduction pathway.

RESISTANCE TO BIOTIC AND ABIOTIC STRESS

The significantly important diseases affecting proso millet cultivation are fungal diseases head smut (*Sporisorium destruens*), kernel smut (*Ustilago crameri*) and bacterial stripe disease (*Pseudomonas avenae*). Apart from diseases, crop destruction by shoot fly (*Atherigona pulla*) in also prominent in India and other growing countries (Lyon *et al*., 2008). Breeding for resistance to head smut in Ukraine identified resistant lines and further led to release of smut resistant varieties namely Kh22 and Kh86 (Konstantinov *et al*., 1991). The base work on head smut by Zhou *et al.* (2016) identified diversity in isolates of *Sporisorium destruens*. Their work identified three pathotypes in isolates and 10 differential hosts in proso millet for pathotype identification. The incidence of shoot fly on proso millet was found to be highest in *kharif* sown crop. Variety GPUP21 was identified to be susceptible to shoot fly infestation (Sathish *et al*., 2017). Baltensperger *et al.* (1995) identified cultivar Huntsman to be resistance to Russian wheat aphid.

The agro-ecosystems of proso millet in several countries require of tolerance to drought and salinity in crop. Response of proso millet genotypes to drought was studied by Seghatoleslami *et al.* (2008). The study identified varieties KCM4 and KCM9 to be suitable for drought affected regions. The drought effect resulted in reduction of seed per ear and seed test weight. Direct selection of promising germplasm in proso millet have been a best approach for identifying potential well performing lines for abiotic stress in proso millet. The crop is more susceptible to water stress than sorghum. Matsuura *et al.* (2012) reported reduction in yield due to reduced grains per panicle under water deficit conditions. Selection indices based on biochemical reaction to stress such as antioxidative enzymes like superoxide dismutase (SOD) and peroxidase (POD) identified 14 accessions having high osmoregulation and thus high degree of drought tolerance in proso millet (Karyudi and Fletcher 2002; Zhang *et al*., 2012). Suppressive subtraction hybridization technique identified 32 ESTs to be involved in response to abiotic stress (Lin *et al*., 2006). Besides these, *S*-adenosylmethionine synthase expression was reported to decline in drought conditions which in turn increased after rehydration. This gene was predicted to play role in water use efficiency.

CONCLUSION AND FUTURE SCOPE

Proso millet is considered ‘miracle grain’ in semi-arid regions considering it potential role in food energy malnutrition. Proso millet comes with numerous health benefits upon its adoption such as preventing risk of cardiovascular diseases. Information on germplasm resources and genomics breeding aspects in proso millet promisingly reduces gap in research outcomes and implementation as inclusion into food-diet. The growth in genomic research accelerates the genetic gain and assist developing high yielding proso millet varieties.

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Conflict of Interest. None.

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