Genetic resources of barnyard millet: enhanced utilization in breeding programs

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ABSTRACT

Policy interventions that promote food diversification aim to supply and provide access to nutrient rich foods apart from usually consumed staple crops. Small millets hold promise in ensuring food diversity and minimise food-energy malnutrition. Being good source of nutrients, they are the future of snack industry considering rise in health-conscious consumers. Barnyard millet is used for food and fodder. End use application of barnyard millet could be enhanced by information on genomic resources and germplasms available for application of breeding practices.

Key words: Genetic resources, Pre-breeding, Small millet, Barnyard millet.

Introduction

Barnyard millet (Echinochloa sp.) is small seeded cereal millet mostly cultivated in Pakistan, India, Japan, Nepal, China and central Africa (Gowda et al., 2009). The Indian barnyard millet (Echinochloa frumentacea) was domesticated in India while the Japanese barnyard millet (Echinochloa esculenta) was originated in eastern Asia. Both species are similar in many traits with overlapping features (Joshi et al., 2015). However, awned and large spikelets with papery glumes makes differs Japanese barnyard millet from Indian barnyard millet. The races of Echinocloa esculenta include crusgalli, macrocarpa, utilis and intermedia. Barnyard millet is rich in protein (>10%), crude fibre and iron (>10 mg per 100g) (IIMR, 2018).

Prebreeding, Germplasm Screening and Selection

Morphological differences are observed in the cultivated species of Echinocloa. They include high seed shattering behaviour of colona and frumentacea while it is low in other two species (Seetharam et al., 2003). Differences are also observed in colour of caryopsis with straw brown colouration getting darker in crusgalli and yellow brow in esculenta. Plant height varies among species with tallest height of 250 cm achieved by crusgalli and frumentacea, colona usually at 60 cm while esculenta reaching intermediate heights (Joshi et al., 2015).

Considering region-wise distribution, majority collections of barnyard millet are conserved in Asia (India and Japan) of which the Indian ICAR-NARS national collections and ICRISAT collections are 4734 and 749, respectively (Table 1). The germplasm collections of Echinocloa species totals to 11268 (http://apps3.fao.org). Core collections in barnyard millet have been established from assessing diversity in more than 700 accession and identifying a core sets of 89 and 50 representative of worldwide collections (Gowda et al., 2009; Upadhyaya et al., 2014). The prebreeding reports state that F1 hybrids derived from cross of two barnyard species are sterile however hybridisation within the genus was reported to be rampant which gave rise to current diversity (Yabuno, 1962; Hili, 1994; Yamaguchi et al., 2015).
The intensive rice-wheat cultivation have sharply decreased production of barnyard millet in India and the world. This reduced diversity of the crop and negligence of its landraces (Sood et al., 2015). Similar to the minor crops, fewer research and resources were devoted to study of barnyard millet. The recent increase in health conscious consumers have recognised the importance of barnyard millet and other small millets owing to nutritional benefits (Varshney et al., 2010). Preliminary morphological characterisation studies of barnyard millet germplasm provides basic information for utility in crop improvement. The Himalayan regions of India harbors promising genotypes for traits like plant height, inflorescence length, tiller number and grain yield (>16g) (Gupta et al., 2009). Germplasm of 89 accessions representing core collections were grouped in three for morpho-agronomic traits (Sood et al., 2015). The studies on barnyard millet germplasm evaluation reported considerable variation for yield related traits (IIMR, 2018). Characterisation of barnyard millet landraces identified resistant lines for grain sumt (RBM 57, RBM77and RBM58), high protein (NDS6, NDS2), high lysine (NDS6, NDS5) and low phytic acid and tannin (NDS6, NDS3).

**Varieties, Mating and Methods**

The breeding objectives primarily are for higher yield, nutritional value, easy de-hulling and climate resilience. The focus has also shifted to traits like early ground cover, stay-green trait, chlorophyll retention. These traits contribute greatly to drought stress. Crossing and hybridization in barnyard millet is a difficult task due to small size of inflorescence (Seetharam et al., 2003). The African and Indian germplasm have been used in recombination breeding which led to development of many varieties of small millets (Seetharam et al., 2003). Fewer barnyard millet genetic resources are registered in India. The registered mostly consist of easy de-hulling trait as in the case of genotype B29. (Gupta et al., 2014). The first variety released was K1 in India. Of the several varieties released in India, notable varieites are PRJ1, MDU1, Co2, VL207, VL-Madira-172 and Gujarat Banti-1 (Table 2). Till 2012, selection from landraces population have facilitated in release of 9 varieties of which 3 were by hybridization (https://www.millets.res.in/aicrp_small.php).

**Molecular Breeding and markers developed**

The development of advanced generation genome sequencing approaches enriched better understanding of genome composition and molecular predictions in crop plants. Chloroplast genome sequencing of seven *Echinochloa* species provided basic information regarding the conserved gene order, genome organization and its structure (Ye et al., 2014; Perumal et al., 2016). The size of chloroplast genome ranged from 139592-139851 bp with an identity of 99.5% among them. Sebastin et al. (2019) reported presence of 61% AT rich regions and 38.6% GC rich regions with a maximum of 131 genes reported in its chloroplast genome. The comparative genomic studies releaved that barnyard millet is closer to *Panicum virgatum* with high sequence identity among them than *Oryza* and *Triticum*.

Randomly amplified polymorphic DNA (RAPD) have been previously used in barnyard millet diversity analysis (Rutledge et al., 2000). The reports when further validated revealed low representation of actual diversity present in barnyard millet germplasm by the usage of RAPD markers (Prabha et al., 2010). The application of sequence based markers is limited in barnyard millet in the current age of advanced genomics. Nozawa

<table>
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<tr>
<th>Crop</th>
<th>Name</th>
<th>ICAR-NARS</th>
<th>ICRISAT</th>
<th>Other</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Barnyard millet</td>
<td>4734</td>
<td>749</td>
<td></td>
<td></td>
<td>1266</td>
</tr>
</tbody>
</table>

Reference:
- Ye et al. (2014)
- Perumal et al. (2016)
- Sebastin et al. (2019)
- Rutledge et al. (2000)
- Prabha et al. (2010)
- Nozawa (2020)
et al. (2006) used five SSR markers and characterised 155 accessions of *Echinochloa*, grouping the accessions of three species into separate 3 clusters. EST-SSR sequences from used from weedy species of *Echinochloa* are less effective for application in cultivated species. A study reporting *in-silico* identification of 22 EST-SSR primers by Babu and Chauhan (2017) also identified SSR repeats of which GT dimers, AGA trimers and TGTTT pentamer sequences were most common. In addition to this, sequencing based identification of SSRs by Chen et al. (2017) in *E. phyllopogon* identified putative SSRs and SNPs. These markers when validated would be of significant utility in studying and understanding diversity, genome structure, mapping of traits, further developing of molecular.

**Genes mapped, QTLs identified**

The linkage maps constructed in barnyard mark the initial steps to understand markers trait association (MTAs) (Wallace et al., 2015; Chen et al., 2017). Ishiwaka et al. (2013) identified MTAs for waxy trait using functional SNPs. They identified three presence of mutant three alleles for waxy gene in barnyard millet namely EeWx1, EeWx1, and EeWx3 associated with low amylose content. In another report of MTA, Renganathan et al. (2019) reported MTA of anthocyanin pigments to SSR marker BMESRR 39. The independent validations of these marker trait associations can be useful in identifying linked markers for utilisation in marker assisted selection.

**Resistance to Biotic and Abiotic Stress**

The barnyard millet cultivation is majorly threatened by grain smut (*Ustilagopanici frumentacea*), loose smut (*Ustilagotritici*) sheath blight and shoot fly. Identification of superior genotypes with resistance to pests is promising approach in millet production. Grain quality and yield is reduced in smut infestation. Joshi et al. (2015) studied grain smut in 49 Indian landraces of barnyard millet and reported susceptibility index of 0.0 to 15.3 with a mean of 8.45. Highest variation was observed for grain smut indicating presence of resistance lines (RBM57, RBM77, RBM78, RBM81 and RBM82). Besides these, Nagaraj and Mantur (2008) and Gupta et al. (2010) identified barnyard accessions resistance to both loose smut and grain smut. Rawat et al. (2019) identified resistant genotypes DHBM996 and TNEF204 against shoot fly and stem borer.

Resistance to abiotic stress for crops like barnyard millet is pivotal importance considering the cultivation soil and agro-ecosystems. *Echinochloa* species usually have higher water use efficiency and dry matter production compared to other millets (Zegada-Lizarazu and Iijima, 2005). Accessions like CO(Kv)2, TNEF204 and MDU 1 were identified to be tolerant to saline growth conditions (Arthi et al., 2019). However, studies on characterization of genes related to performance to abiotic stress are yet to be done. The molecular mechanism pathways are to elucidate. Moreover, breeders are focussing on stay-green trait, early ground cover, chlorophyll retention at later stages to combat moisture stress.

**Conclusion**

The health benefits associated with food diversification with small millets in incredible. Barnyard millet, being an underutilised crop, has tremendous scope of improvement through application of

<table>
<thead>
<tr>
<th>Varieties</th>
<th>Remarks</th>
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<tbody>
<tr>
<td>Co2</td>
<td>Pureline selection from EF79</td>
</tr>
<tr>
<td>RAU11</td>
<td>Pureline selection (Sushrutha)</td>
</tr>
<tr>
<td>VL207</td>
<td>Pedigree selection VL172xGECH506</td>
</tr>
<tr>
<td>Co(KV)2</td>
<td>Good grain quality</td>
</tr>
<tr>
<td>GP70A</td>
<td>Grain smut resistant</td>
</tr>
<tr>
<td>GP90A</td>
<td>Grain smut resistant</td>
</tr>
<tr>
<td>GP110</td>
<td>Grain smut resistant</td>
</tr>
<tr>
<td>GP117</td>
<td>Grain smut resistant</td>
</tr>
<tr>
<td>PRJ1</td>
<td>Grain smut resistant</td>
</tr>
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Table 2. Details of famous barnyard millet varieties in India
genomics assisted breeding. In this international year of millet, improvement of major and minor millets can potentially improve germplasm for targeted traits and thus provide better food diversification source.

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All authors contributed equally

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**Conflict of Interest**

The authors declare that no conflict of interest exists.

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