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Genetics, breeding, and genomics of Indian barnyard millet (Echinochloa frumentacea): status and perspectives

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1. Introduction

Despite the substantial rise in agricultural production in the second half of the twentieth century, just over 12 crops account for 75% of global food resources, and three primary crops, rice, wheat, and maize, accounting for 50% of global food choices (Joshi et al., 2018). The narrow agricultural portfolio raises serious questions on how effectively major crops alone can contribute toward food security and poverty alleviation. Although the fact that these main staple cereal grains are an integral part of many people's diets, they have an unbalanced nutritional profile mainly in terms of essential minerals and protein quality. Besides this, existing cropping systems are also becoming vulnerable to climate change due to their overdependence on few crops (Joshi et al., 2019). To address this issue, efforts must be expanded to incorporate diverse crop species into modern cropping systems. Supplementing major cereals with small millets possessing higher nutritive value and nutraceutical qualities may be a potential strategy for increasing food diversity and building the climate resilience of the modern agroecosystems (Kumar et al., 2021).

Millets are ancient supergrains belonging to the family Poaceae. Among the small millets, barnyard millet (*Echinochloa* spp.) is one of the oldest cultivated crop species. Barnyard millet is a versatile crop that can be used for both food and fodder. In recent times, it has become important due to its noble nutritional composition, recognized health profits, versatile environmental adaptability, viability to marginal agriculture, and organic cultivation (Sood et al., 2020). Barnyard millet in India is predominantly grown under two

varied agro-ecological conditions, one in the Northern part of the country under the lower and mid-hills of the Himalayan region and the other in the southern part mainly in the Deccan plateau region (Sood et al., 2016).

The genus *Echinochloa* comprised two cultivated species viz. *Echinochloa frumentacea* and *E. esculenta*. *E. frumentacea* is known as Indian Barnyard millet, and *E. esculenta* is known as Japanese barnyard millet (Sood et al., 2015a). Both cultivated species, as well as their progenitors, are hexaploid with $2n = 6x = 54$, where $x = 9$. The Indian barnyard millet is cultivated in India, Nepal, China, Malaysia, and Africa and consumed as a substitute for rice (Anuradha et al., 2014). It has the fastest growing character among all the millets and is well adapted to fragile and hilly mountain ecosystems (Gururani et al. 2021). At present, India is the largest producer of barnyard millet, both in terms of area (0.146 m ha) and production (0.147 mt) (IIMR, 2018).

2. Phylogeny, origin, and history

The genus *Echinochloa* belongs to the family Poaceae and subfamily *Panicoideae* and includes 250 described annual and perennial species, of which *E. frumentacea* (Indian barnyard millet) and *E. esculenta* (Japanese barnyard millet) are the most important and widely cultivated (Clayton and Renvoize, 2006). All the species of genus *Echinochloa* have wide adaptation and scattered throughout the warmer and temperate regions of the globe (Bajwa et al., 2015).

Echinochloa frumentacea (Roxb.) Link; syn. *E. colona* var. *frumentacea* is generally acknowledged as Indian barnyard millet and thought to have originated from its wild hexaploid ($2n = 6x = 54$) progenitor *E. Colona* (Hilu, 1994). The wild barnyard millet (*Echinochloa colona*) is a common weed of rice fields, and in several parts of India, it is used as a food source during extreme drought spells (Padulosi et al., 2009).

Indian barnyard millet is one of the oldest domesticated crops and archaeological records of its cultivation date back to 5000 B.C. in India and 3000 B.C in China (Doggett, 1989). Based on the morphology of inflorescence, *E. frumentacea* has been classified into four races, namely, *stolonifera*, *intermedia*, *robusta*, and *laxa*, which are widely distributed in India, Nepal, China, Central Africa, Malawi, and Tanzania (Upadhyaya et al., 2014).

3. Botanical description and floral biology

The *E. frumentacea* plant is an annual herb growing 1.0–2.2 m tall characterized by slender to strong culm (Padulosi et al., 2009). In general, plants are green in color, but there are violet tinges in the somatic as well as sexual parts. The plants are generally characterized by smooth, glabrous, and liguleless leaves with slight hairiness and an arc of 15–40 cm long and 1–2.5 cm wide (Sood et al., 2020) (Fig. 5.1).

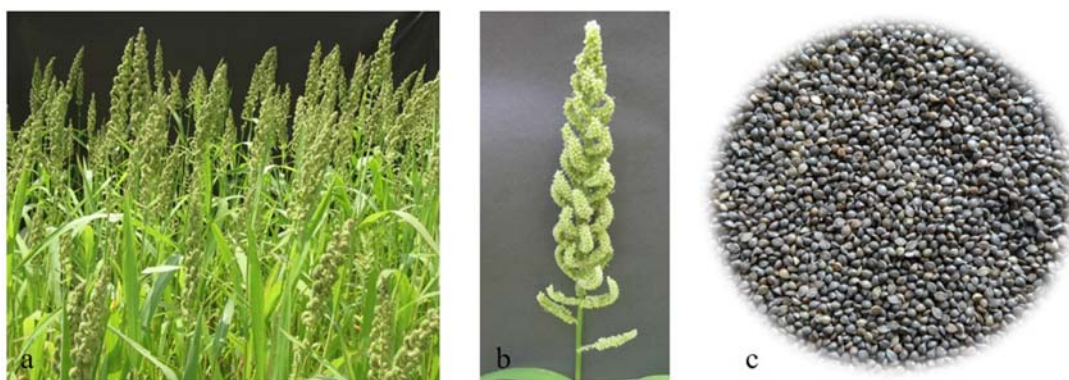


Figure 5.1

Barnyard millet plants (A), inflorescence, (B) and grains (C).

The inflorescence is a typically erect and terminal panicle (10–28 cm long) of green to violet color, with numerous and 1–3 cm long racemes, seldom drooping, and mostly awnless (Fig. 5.1). The spikelet present on the panicle is small, unbranched, densely packed in three to five rows, 2–4 mm long, acute, and awnless on the rachis (Renganathan et al., 2020). The spikelet is occupied with stiff bristles and congested. The spikelets are subsided by two glumes within which there are two florets. The upper floret is bisexual, while the lower floret is sterile. The color of the glume varies from white to red. There are three stamens present, and two distinct types of styles each with plumose stigma are found on the superior ovary (Sundararaj and Thulasidas, 1976).

The blooming begins at the inflorescence apex and progresses down and usually takes 10–15 days to complete. Anthesis starts from 5 to 10 a.m., while most of the flowers open between 6 and 7 a.m. (Sundararaj and Thulasidas, 1976; Jayaraman et al., 1997; Gupta et al., 2012). Flowering begins first at the marginal ends of each raceme and progresses to the individual raceme's center. The stigmatic branches extend and the hermaphrodite flower opens afore the anthers dehiscence (Seetharam et al., 2003). *E. frumentacea* has smaller, awnless spikelets, with membranous glumes, whereas *Echinochloa esculenta* has large generally awned spikelets with cretaceous upper glumes and lower lemma (Yabuno, 1987). Contact method is the traditional hybridization method followed in barnyard millet. However, growing all seeds harvested from female panicle for identification of few hybrid plants in contact method requires more resources time, space, and labor (Sood et al., 2020). Hot water treatment of inflorescence at 48°C for 4–5 min was also found effective for the emasculation under hill conditions (79°39' E and 29°35' altitude 1250 m amsl) in barnyard millet (Parihar et al., 2021). In contrast to the contact method, only few seeds set in the female panicle in hot water treatment, most of which give rise to hybrid plants. Grain is around 2–3 mm in length and 1–2 mm in width and bounded by hardened lemma and palea with white shining. The grain color is generally yellow or white.

4. Growth, development, and agronomic attributes

Barnyard millet is an important crop for famine areas owed to its rapid growth, drought tolerance, and capability to thrive well under marginal growing conditions (Majid et al., 1989). Although, it can be cultivated in a wide range of growing conditions but requires a warm and moderately humid climate for abundant growth and yield. In general, the crop is sown from the second fortnight of June to the first fortnight of July with the onset of monsoon rains. Planting late lessens the growth period and adversely affects the crop yield. Generally, *E. frumentacea* takes about 70–90 days to complete its life cycle, but under unfavorable circumstances, it may take more time to complete its life span. Owing to its quick growth and short duration, it is an ideal catch crop. Barnyard millet grows under natural precipitation and does not require irrigation facilities.

It favors sandy soil with good drainage, having 4.6–7.4 pH values but has also been adapted to flooded marshland, marginal grounds, and acidic soils with a low pH value of 4.5 and electric conductivity of 3.12–4.68 dS m⁻¹ (Farrell, 2011). It can be used as retrieve crops in a field that is else saline for rice cultivation. The ideal temperature range for optimal growth of *E. frumentacea* is 27–33°C during the day and 15–22°C at night (Muldoon et al., 1982). While low-temperature conditions (15/10°C day/night) stop the growth and have an impact on plant production, largely on leaf area and dry weight traits.

Under favorable growing conditions, the crop seeds germinate within 24 h of water imbibition and plants' adventitious roots emerge after 5 days of germination (Rahn et al., 1968). The first tillers appear about 10 days after emergence (Holm et al., 1991). The plants exhibit abundant vegetative growth from 20 to 25 days after emergence, and subsequently, the switch from vegetative to reproductive phase takes place quickly in less than 40 days after emergence (Maun and Barrett, 1986). After emergence, the seed filling period starts in the 55–60 days. After the inception of the reproductive process, seeds mature within 20–40 days. Barnyard millets should be harvested within 10–12 weeks after sowing when 70–75% of the seeds have attained physiological maturity. For drying purpose, the ideal temperature and moisture content are about 45 °C and 16%, respectively. Indian barnyard millet has been reported as a quantitative short-day plant (Muldoon, 1985). Plants are small and flower quickly but abundantly under short-day conditions, whereas, under long-day conditions, the plants are more vigorous with a high seed yield (Maun and Barrett, 1986; Manidool, 1992). However, most *Echinochloa* spp. can grow and reproduce in a wide range of photoperiods, including short (8–13 h) and long (16 h) days (Maun and Barrett, 1986; Mitich, 1990).

The nutrient requirement of barnyard millet is fairly low as compared with major cereals. Farmyard manure or compost at the rate of 5–10 tons per hectare is sufficient for enhancing productivity. Along with organic manures, the crop with 40 kg N₂, 20 kg P₂O₅ and 20 kg K₂O per hectare or as per soil testing recommendations. All the fertilizer should

be added to the soil during sowing time. But, if irrigation facilities are available, half of the nitrogen dose should be top-dressed in a standing crop after 25–30 days of sowing.

5. Nutrition composition and nutraceutical potential of barnyard millet

Barnyard millet is a decent source of high-quality digestible protein with the least caloric compactness in comparison to major cereals. Barnyard millet is highly nutritious, comprising about 55% starch, 11% protein, 3.9% fat, and 13.6% crude fiber content, as well as with noteworthy content of calcium and iron (Saleh et al., 2013). The average nutritional composition of barnyard millet in comparison to other major cereals based on per 100 g edible portion is furnished in Table 5.1.

Table 5.1: Nutritional composition of barnyard millet (per 100 g dry weight basis) in comparison to major cereals.

Nutrients	Barnyard millet	Rice	Wheat	Maize
Proximate composition (per 100 g grain)				
Carbohydrate (g)	65.5	78.2	69.4	66.2
Protein (g)	11.6	6.8	12.1	11.1
Fat (g)	5.8	0.5	1.7	3.6
Fiber (g)	13.5	0.2	1.9	2.7
Mineral (g)	4.4	0.6	2.7	1.5
Mineral elements (mg per 100 g grain)				
Iron (mg)	5	0.7	4.9	2.3
Calcium (mg)	20	10	48	10
Zinc (mg)	3	1.4	2.2	2.8
Phosphorus (mg)	280	160	355	348
Magnesium (mg)	82	90	132	139
Essential amino acids composition (mg per g protein)				
Isoleusine	288	181	148	154
Leusine	725	345	269	480
Lysine	106	166	130	126
Methionine	133	103	56	85
Cysteine	175	101	101	97
Phenylalanine	362	222	179	199
Tyrosine	150	199	125	166
Threonine	35	144	125	148
Tryptophan	63	55	43	16
Valine	388	269	199	211

Based on Sources: FAO, 1995. *Sorghum and Millets in Human Nutrition*, FAO Food and Nutrition Series No. 27. Food and Agricultural Organization, Rome, Italy; Gopalan, C., Ramshashtri, B.V., Balasubramanian, S.C., 1999. *Nutritive Value of Indian Foods*, second ed.; Narasinga Rao, B.S., Deosthale, Y.G., Pant, K.C. (ed.) National Institute of Nutrition, Indian Council of Medical Research, New Delhi; Gopalan, C., Rama Shastri, B.V., Balasubramanian, S.C., 2009. *Nutritive Value of Indian Foods*, National Institute of Nutrition, Indian Council of Medical Research, Hyderabad, p 99.

The carbohydrate content of barnyard millet is slowly digestible, making it a natural gift for people suffering from diabetes (Veena et al., 2005). It has also more fiber and iron content than barley, wheat, and other millets. The high fiber content benefits in inhibiting digestion problems such as constipation, excess gas, bloating, and cramping (Rao and Bhaskarachary, 2017). These features make it a worthy contestant for the preparation of industrially processed food products like baby foods, snacks, and dietary food products (Ugare, 2008; Vijayakumar et al., 2009; Anju and Sarita, 2010; Surekha et al., 2013).

Barnyard millet grains are dehusked and baked in the same way as rice grains. Barnyard millet in the Himalayan region is consumed as *paleu* or *chenna*, a savoury cooked porridge made with buttermilk. Whereas, in some parts of South India, the grain is processed very similar to rice parboiling (Bhat et al., 2019) and used in *Idli*, *dosa*, *chakli*, etc. Besides these, the biscuits, cakes, sweets, pasta, rusk, ready mix, popped products, and other specialty food products have all been made with barnyard millet (Arora and Srivastava, 2002; Poongodi et al., 2003).

Barnyard millet grains are rich reservoir of flavonoids such as luteolin and tricetin, which are known to have anticarcinogenic, anti-inflammatory, antioxidant, and antileukemic properties (Han et al., 2007; Shahidi and Chandrasekara, 2013; Rao et al., 2017). Immature seeds are known to possess diuretic properties and improve virility strength (Chauhan and Johnson, 2011). It is an ideal food for those patients who have gluten intolerance and celiac disease. The low glycemic index of barnyard millet makes it an excellent diet for type II diabetic patients (Sharma et al., 2013). Other functional constituents of barnyard millet grains include γ -aminobutyric acid and β -glucan, which act as antioxidants and help to lower blood lipid levels (Sharma et al., 2016). There is an inevitability to advance millet-based foodstuffs in the formulation of ready-to-use foodstuffs and functional nourishments to fulfill the requirements of the prevailing-day users. Value addition does not merely deal to provide diversity, handiness, and value diet to consumers; however, it is also vital for the resurgence of barnyard millet production (Sood et al., 2015a).

6. Adaptation to climate change and local environments

By virtue of its adaptation to low input agriculture and harsh weather patterns, barnyard millet is well adapted to fragile mountain agro-ecosystem. The short life cycle (80–90 days), dense tap root system, thickened cell walls, C4 photosynthesis system, and large leaf area index with high radiation use efficiency barnyard millet makes barnyard millet an ideal crop for future agricultural systems.

7. Intercropping systems

Barnyard millet is mostly grown as a pure crop, followed by pulses like chickpea and cereals (wheat and barley). In Uttarakhand hills of India, barnyard millet is mostly grown

as an intercrop with rice bean, black soybean, and horse gram. The profitable barnyard millet intercropping system in hills has been found to be with soybean/rice bean in the ratio of 9:1 (Shalini et al., 2019). The number of studies on intercropping in barnyard millet is limited, and there is a need for extensive research on barnyard millet-based intercropping systems.

8. Bottlenecks in barnyard millet breeding

Barnyard millet is predominantly self-pollinating (Potvin, 1986) and self-compatible crop species. Because of the small size of the flowers, the early hours of flowering, the limited pollen viability duration, the nonavailability of the pollen grain, and the short period of flowers opening, emasculation, and artificial hybridization are challenging processes (Nirmala Kumari and Vetriventhan, 2009). Therefore, the success rate of making the cross is usually very low restraining the number of genetic studies and yield advancement in barnyard millet. As a result, most of the present-day's barnyard millet cultivars are the product of traditional mass selection and pure-line selection, and recombination breeding is still a daunting challenge (Joshi et al., 2021). Comprehending the floral biology and pollination pattern will aid in inventing suitable emasculation and crossing procedures (Gupta et al., 2012).

Barnyard millet grain requires dehulling before making it suitable for human consumption (Lohani et al., 2012). Therefore, another important breeding goal for reducing the drudgery of postharvest processing of barnyard millet is to be bred for easy dehulling type genotypes. Some researchers reported the occurrence of easy dehulling genotypes in local germplasm (Gupta et al., 2015), which may be utilized in breeding programs. Since barnyard millet is generally eaten like rice, therefore, the increased grain size is expected to increase market demand. Although, this rests a problem due to the trait's limited spectrum of variability. Besides these, nonshattering behavior is also very critical for yield improvement via reducing yield losses.

9. Major biotic and abiotic production constraints

The genus *Echinochloa* can withstand a variety of biotic and abiotic stresses with minimum yield losses. Although, cultivated species *E. frumentacea* is usually vulnerable to many biotic stresses mainly pests and diseases at different stages during crop growth (Jain et al., 1997; Jagadish et al., 2008). Therefore, the major breeding target traits to overcome biotic constraints in barnyard millet are resistance to grain smut, loose smut, sheath blight, shoot fly, stem borer, and aphid. Barnyard millet grain smut (Fig. 5.2) that is caused by *Ustilago panici-frumentacei* Brefeld is the main productivity constraint resulting in yield reduction up to 60.8% (Jain et al., 1997).



Figure 5.2

Smut in barnyard millet (A) grain smut (B) head smut. Source: Reproduced from Sood, S., Khulbe, R.K., Gupta, A.K., Agrawal, P.K., Upadhyaya, H.D., Bhatt, J.C., 2015a. Barnyard millet - a potential food and feed crop of future. *Plant Breed* 134:135–147. <https://doi.org/10.1111/pbr.12243> with permission.

The flower ovaries of infected panicles are round and hairy, and they might expand 2–3 times than their normal size. Gall-like swellings can occasionally be seen on the nodes, axils of older leaves, and the stem. Likewise, *Ustilago crus-galli* Tracy and Earle is responsible for head smut condition (Nagaraja et al., 2007). This happens near the end of the growing season when the crop is about to mature.

The resistance source for grain smut has not been recognized in Indian barnyard millet, whereas Japanese barnyard millet confers strong resistance against it (Joshi et al., 2021). However, due to strong compatibility barriers between the two species, it is difficult to transfer resistance from Japanese barnyard millet to Indian barnyard millet (Sood et al., 2014). The alternate source for the improvement of Indian barnyard millet could be *E. colona*, the wild progenitor that is resistant toward grain smut and crossable with cultivated species. Loose smut caused by *Ustilago tritici* fungus is also the main disease that is responsible for affecting the grain development in both the cultivated species of *Echinochloa* genus (Jain et al., 1997; Gupta et al., 2010a).

On the other hand, Nagaraja and Mantur (2008) demonstrated that some *E. esculenta* accessions were immune to all smut diseases, allowing for the breeding of resistance lines. Gupta et al. (2010a) tested 257 barnyard millet accessions for grain smut tolerance, including advanced breeding lines, and clustered accessions based on response to smut infection, identifying highly resistant accessions. Similarly, *Helminthosporium* leaf blight,

which is a major concern in certain regions, should also be a focus of resistance breeding efforts (Jain et al., 1997).

Among the insect pest, pink stem borer (*Sesamia inferens*) (Fig. 5.3) and shoot fly (*Altherigona falcata*) are the most major insect pests of barnyard millet (Jagadish et al., 2008). The pink stem borer tunnels the stem resulting in dead heart/whitehead formation, while the shoot fly causes tunneling in the nursery stage. Therefore, *E. frumentacea* suffers a significant yield loss due to stem borer and shoot fly infection during the vegetative period.

In India, some promising germplasm accessions and barnyard millet varieties have been identified to be comparatively resistant to shoot fly (Murthi and Harinarayana, 1986). Subsequently, shoot fly and stem borer resistant genotypes have been identified in barnyard millet as DHBM 996 and TNEF-204 (Rawat et al., 2019).



Figure 5.3

Stem borer and its damage in barnyard millet. Source: Reproduced from Sood, S., Khulbe, R.K., Gupta, A.K., Agrawal, P.K., Upadhyaya, H.D., Bhatt, J.C., 2015a. Barnyard millet - a potential food and feed crop of future. *Plant Breed.* 134:135–147. <https://doi.org/10.1111/pbr.12243> with permission.

The pink stem borer tunnels the stem, culminating in the creation of a dead heart/white head, whereas the shoot fly tunnels the stem in the nursery stage. The crop has a better storage life and keeping quality, and it is pest-free.

Barnyard millet is well-adapted to abiotic stresses compared with most other cereal crops (Gupta et al., 2010b; Singh et al., 2010; Dwivedi et al., 2012). However, its production is hampered by the lodging, resulting in significant yield and quality losses. Under the high moisture conditions that prevail during the rainy season, barnyard millet can reach a height of 2 m or more making it vulnerable to lodging. This problem could be mitigated by reducing plant height 120 to 130 cm. The resulting decrease in fodder yield due to reduced height could be offset by creating genotypes with more number of basal tillers, which would increase grain yield as well. The length and number of spikes in barnyard millet are positively associated with grain yield. As a result, developing lodging-resistant cultivars with more and longer spikes in combination with good crop husbandry could be the common approach for increasing grain yield.

10. Breeding advancements in barnyard millet

10.1 Conventional breeding strategies

In India, barnyard millet breeding gained interest at the national level in 1969, with the inception of the All India Coordinated Millets Improvement Project. Subsequently, five crop-specific lead research centers in the country were established with International Development Research Center (IDRC) assistance during 1978–79 providing a significant boost to small millets improvement (Seetharam, 1998). Among them, ICAR-VPKAS, Almora in Uttarakhand, was nominated for barnyard millet improvement. Conventional breeding strategies, which include pure line selection, pedigree selection, mass selection, and mutation, that are pertinent to other self-pollinating crops are also equally applicable in barnyard millet. In barnyard millet, most cultivars (about 18 in number) in India were released ensuing pure-line selection from local landraces/cultivars, accompanied with the aid of pedigree selection (Table 5.2) (AICSMIP, 2014).

10.2 Mutation breeding

Mutation breeding has played a key role in self-pollinated crops, where hybridization is challenging to achieve variability (Sood et al., 2019). Mutation breeding was started to induce genetic polymorphism for yield traits. Inducing a mutant phenotype in polyploid species like *Echinochloa* is very challenging. Gamma irradiation treatment is reported to improve genetic variability for the seed yield and yield components such as the number of tillers, plant height, ear head, and length (Mehra et al., 1985).

Table 5.2: Improved and popular cultivars of Indian barnyard millet developed in India (Sood et al., 2020).

S. No.	Name of cultivar	Silent features
1.	VL <i>Madira</i> 172	Average grain yield is 2.2–2.3 t ha ⁻¹ . Mature in 75–80 days. Tolerant to grain smut and well suited to high cropping intensity.
2.	VL <i>Madira</i> 181	Average grain yield 1.6–1.7 t ha ⁻¹ . Mature in 90–95 days. Profuse tillering.
3.	VLM <i>Madira</i> 207	Average grain yield is 1.6–1.9 t ha ⁻¹ . Mature in 80–90 days. Pyramidal shaped panicles. Tolerant to grain smut. Also having improved plant type with high harvest index.
4.	CO(KV) 2	Average grain yield is 2.1–2.2 t ha ⁻¹ . Mature in 95–100 days. Having branched panicles, nonlodging, profuse tillering, and suitable for contingency planting.
5.	ER 64 (Pratap <i>sawan</i> 1)	Dual-purpose variety giving fodder yield 5.0–5.5 t ha ⁻¹ and average grain yield is 1.5–1.7 t ha ⁻¹ . Resistant to smut and tolerant to shoot fly
6.	RAU 11 (<i>Sushrutha</i>)	Average grain yield is 2.0–2.2 t ha ⁻¹ . Erect plant type, compact panicles. Profuse tillering, high yielding, and very early duration (75–80 days).
7.	DHBM 93-3	Average grain yield is 2.2–2.4 t ha ⁻¹ . Mature in 90–95 days. Responsive to fertilizer application

11. Modern breeding approaches for genetic improvement

11.1 Genetic diversity studies through molecular markers

Initially, Hilu (1994) employed the random amplified polymorphic DNA (RAPD) markers to exploit the genetic divergence and phylogenetic relationship between *Echinochloa* species and revealed that RAPD markers were working to differentiate at the genomic level both the cultivated as well as wild progenitors of the *Echinochloa* species. *E. frumentacea* species was shown to be more genetically diverse than *E. utilis* populations. To solve the problem of the low magnitude of polymorphism by RAPD markers, amplified fragment length polymorphism (AFLP) markers were employed to investigate the genetic divergence in *Echinochloa* species (Danquah et al., 2002; Tabacchi et al., 2009). The microsatellites and polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) were also employed to establish species relationships in genus *Echinochloa* (Danquah et al., 2002; Yamaguchi et al., 2005; Yasuda et al., 2006). Likewise, in *E. colona*, *InDel* markers were used for recognition of *rbcl*, *matK*, and *ITS* genes and also had been extensively used as a low-cost method for barcoding of the DNA in *E. colona*, *E. oryzicola*, and *E. crus-galli* species (Lee et al., 2014). Besides SSR markers, the genotyping-by-sequencing (GBS) approach has also been used to generate 21,000 SNPs in barnyard millet core collection germplasm (Wallace et al., 2015). Recently, Babu et al. (2018a, b) employed rice, finger millet, and maize genomic SSRs for cross-transferability analysis among barnyard millet cultivated and wild species for identification of

polymorphic markers, syntenic regions, genetic diversity, and population structure analysis. The SSRs of all the crops were highly transferable, polymorphic, and were able to distinguish barnyard millet genotypes clearly.

11.2 Gene/QTL mapping

In barnyard millet, numerous SSR and SNP markers are recognized (Wallace et al., 2015; Chen et al., 2017; Manimekalai et al., 2018; Murukarthick et al., 2019) to expedite the linkage map creation and QTL mapping; however, no genetic linkage map is reported so far. Till now, only two mapping studies are available publicly in barnyard millet; one for waxy traits (Ishikawa et al., 2013) that used functional SNP markers and revealed that three loci, namely, EeWx1, EeWx1, and EeWx3 were responsible for controlling waxy traits. Likewise, Renganathan et al. (2019) employed bulk segregants analysis (BSA) and 51 EST-SSR markers to study the genetics of anthocyanin pigmentation and revealed that the anthocyanin pigments in barnyard millet is closely linked to SSR marker, BMESSR 39. Barnyard millet genome mapping is still in its infancy. Therefore, advance experimental investigation on mapping studies is necessary for the exploitation of marker-assisted selection strategy (Renganathan et al., 2020).

11.3 Genomic resources and utilization

The whole-genome sequencing approach is compulsory to comprehend the genome alignment and gene catalog of a crop and to find out key genes as well as pathways linked to traits of economic importance (Joshi et al., 2021). However, limited genomic information, such as DNA markers, genetic/linkage maps, and genome sequences, is the major constraint for the molecular characterization of barnyard millet crop. The whole genome sequencing has already been completed in other millets like sorghum, pearl millet, foxtail millet, finger millet, and proso millet (Zhang et al., 2012; Mace et al., 2013; Hittalmani et al., 2017; Varshney et al., 2017; Zou et al., 2019). Due to genome complexity and the dearth of exploration effort in barnyard millet, very restricted attempts have been made to ascertain the genomic organization as well as associated advancements. Genomic resources from closely related species where genome sequences are existing could be exploited toward supplementing genomic resources in barnyard millet.

12. Comparative genomics and synteny studies

Comparative genomics plays a critical role by using the synteny evidence between conserved regions among the crops plant belonging to the same family (Moore et al., 1995; Gale and Devos, 1998; Pattanayak et al., 2019). Owing to the scarcity of genome sequence information, comparative genomic studies will be an effective tool for genomic

studies in barnyard millet. A high amount of cross-transferability was revealed in barnyard millet by using the genomic microsatellite markers that were established via in silico mining for foxtail millet (Pandey et al., 2013; Kumari et al., 2013). The 100 intron-length polymorphic markers mined from the foxtail millet genome revealed cross transferability of 94% in Indian barnyard millet (Muthamilarasan and Prasad, 2014). Likewise, more than 70% cross transferability of rice genic SSR obtained from the calcium transporters and calcium kinase primers to Indian barnyard millet was demonstrated (Yadav et al., 2014). These markers would be of tremendous and profound value for studies of genetic diversity, creation of linkage map, and additionally their use in marker recognition for significant agro-morphological traits that are linked to QTLs in barnyard millet. Further, these recognized QTLs will be able to efficiently introgressed through marker-assisted selection in the barnyard millet genotypes that are locally adapted for enhancement of yield and stress regulation.

13. Functional genomics approach for gene discovery

13.1 Transcriptomics

Gene expression profiling based on transcriptome analysis is a powerful functional genomics technique for identifying candidate genes that control a variety of biological processes (Kumar et al., 2016). Enormous transcript profiles for different characters accompanied with invasiveness and adaptations processes like resistance toward herbicides, photosynthesis, flooding, and other genes related to homeostasis, have been developed in the weedy *Echinochloa* species (Li et al., 2013a,b; Yang et al., 2013; Nah et al., 2015; Xu et al., 2015; Guo et al., 2017; Gao et al., 2018). However, transcriptome profiles related to traits of economic importance like disease resistance and nutritional quality are yet to be developed in the Indian barnyard millet.

13.2 Proteomics and metabolomics

Proteomics is a functional genomics technique that infers to the inclusive analysis of a whole set of proteins in terms of organization, expression, and functional properties. The proteomics often employed various techniques such as 2-D gel electrophoresis, mass spectrophotometry, and gel-free shotgun (Matros et al., 2011). The speedy advancements toward proteomics techniques via proteome mapping, comparative proteomics, and the discovery of protein–protein interactions are enabling new perceptions into plant genomes (Varshney et al., 2009). However, the studies using proteomics with major emphasis to comprehend seed quality characters may not yet have been applied in barnyard millet. Metabolomics is a new “omics” technology that involves identifying, characterizing, and quantifying biomolecules having a low molecular weight in a biological environment (Kumar et al., 2016). These low-molecular-weight biomolecules are referring as

metabolomes. Various secondary metabolites such as polyphenols and flavonoids have been revealed to play a significant role in the nutraceutical belongings of barnyard grain. As a result, comprehensive metabolomics studies are needed to conclude the best genotypes for human consumption. However, barnyard millet grain metabolomics profiling is yet to be available.

13.3 Genetic transformation methods

To satisfy the growing global demand for cereals, new methods, and techniques for developing new varieties are needed (Gaspairs et al. 2008; Jagga-Chugh et al., 2011). Development of transgenic plants with predictable traits is one of these effective approaches. In contrast to major cereals, effective transformation protocols are yet to be standardized in small millets including barnyard millet. However, efforts toward the development of a fast, effective, and repeatable regeneration protocol (Rajak et al., 2018) are encouraging efforts toward the standardization of genetic transformation barnyard millet. So far, there is only one report available regarding genetic transformation studies in barnyard millet, which is based on biolistic transformation for evaluating the efficacy of different promoters in GUS expression (Gupta et al., 2001). However, there has been no transformation attempt through the *Agrobacterium*-mediated method in barnyard millet.

14. Conclusion and future outlook

Barnyard millet breeding plans have come to a halt, owing to a lack of support in terms of capital and patronage from different funding and research organizations. Hence, greater efforts are required to evolve cultivars with economic traits that are preferred by the farmer as well as the consumer. In the future, more breeding plans are needed to take advantage of the genetic variability to improve the yield potential, stabilization of yield, salinity tolerance, pest and disease resistance, and superior nutritional worth, primarily micronutrient constitutes (Renganathan et al., 2020). Nevertheless, due to a scarcity of genetic and genomic resources in barnyard millet, the advances in breeding programs are very slow.

Current crop production is challenged especially due to climate change, which has resulted in enlarged drought, salinity, and heat problems (Mabhaudhi et al., 2019). Besides this, agricultural production resulted in an untenable way owing to dependence on expensive chemical fertilizers and pesticides, which are responsible for environmental degradation and increased production costs (Fernie and Yan, 2019). Under such circumstances, the use of underutilized and potential crops like barnyard millet, which can offer diversification for food and genetic resources, is one of the prospective ways to tackle these problems. Due to their diverse adaptation on marginal lands, low input requirements, reduced vulnerability to stresses, and nutritional supremacy as paralleled to main staple cereals.

Increasing consumer's awareness of balanced diets, as well as the climate-resilient properties, highlights the requisite of putting more effort into research for barnyard millet. As there is a scarcity of germplasm and data on genetic multiplicity, their tangible use in crop improvement programs is restricted. Hence, highlighting the germplasm pool is vital for recognizing trait precise resources, genes and alleles, which can be employed in barnyard millet improvement. The yield hindrance in barnyard millet can be smashed through the use of male sterility and manipulating heterosis, and enhancement of genomics-assisted breeding, in conjugation using improved cultivation practices. To speed up barnyard millet improvement, genomics-aided breeding would expedite the credentials of novel alleles and genes with better agronomical efficiency as well as tolerance to biotic and abiotic stresses. To upscale the barnyard millet as a worthwhile crop to farmers, the researchers must need to be characterized available germplasm resources, utilization genetic and genomic tools designed for breeding high-yielding cultivars, and development of improved packages of practices for cultivation, as well as the processing and improving consumption through value addition.

Thus, there is an urgent need to develop a roadmap to upkeep barnyard millet cultivation, through various efforts like the development of distinct cultivars for explicit environments and pursuing attributes upgrading through modern breeding and genomic tools. These strategies would aid in the fighting against starvation and malnutrition and also conjointly benefiting to the farmers and other players tangled in barnyard millet cultivation in the face of climate change.

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Further reading

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