



# Harnessing wild relatives of pearl millet for germplasm enhancement: Challenges and opportunities

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## Abstract

Pearl millet [*Pennisetum glaucum* (L.) R. Br.] is one of the world's hardest warm-season cereal crop and is cultivated mainly in the semi-arid tropics of Asia and Africa for food, feed, fodder, and brewing. It is mainly cultivated for its gluten-free grains with high content and better quality of nutrients. Pearl millet is a resilient crop that can produce grain and biomass under harsh conditions like low fertility, erratic rainfall, acidic and saline soils, and the hottest climates. However, biotic stresses such as downy mildew and blast diseases and abiotic stresses, especially drought and seedling- and flowering-stage heat stress, pose constant threat to the realization of yield potential of this crop. To make further improvement in threshold level of abiotic and biotic stress tolerance, breeders are looking for novel genes in diverse germplasm sources. Crop wild relatives (CWRs) could be a source of novel genes that are important for diversification of the genetic base of pearl millet. A stage-gate process is proposed for the efficient management of prebreeding programs using CWRs as a source of germplasm diversity and improvement. This article explains the various strategies for capturing and using alleles for climate resilience traits improvement. This article covers breeders' perspectives on importance of using CWRs as germplasm source for crop improvement. This article also describes the availability of CWRs, characterization of new traits and the strategies to be applied for the identification and introduction of genes of interest in elite breeding lines and commercial varieties and hybrids of pearl millet.

**Abbreviations:** CMS, cytoplasmic male sterility; CWR, crop wild relative; GP1, primary gene pool; GP2, secondary gene pool; GP3, tertiary gene pool; IL, introgression line; SNP, single nucleotide polymorphism; TGP, trait-specific gene pool; TGS, trait-specific genetic stock; TPE, target population environment.

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## 1 | INTRODUCTION

Pearl millet [*Pennisetum glaucum* (L.) R. Br., syn. *Cenchrus americanus* (L.) Morrone] is an important dryland cereal crop in the arid and semi-arid regions of the world. It is a protogynous, short duration C<sub>4</sub> plant with low chromosome number

( $2n = 2x = 14$ ), high multiplication ratio (up to 1:1000), and excellent ratooning ability. These characteristics make this crop an excellent tool for cytogenetic and breeding research (Burton & Powell, 1968). Pearl millet is cultivated on an area of over 30 million ha in about 30 countries, mainly in Africa followed by Asia (Yadav & Rai, 2013). India is the largest producer of pearl millet, both in terms of area (6.93 million ha) and production (8.61 Tg). A significant shift in pearl millet cultivation from the rainy season to the summer season was observed in Gujarat, India (Reddy et al., 2013) followed by an expansion to large areas (>500,000 ha) in northern and western India. Efforts are also underway in western Asia and northern Africa and western and central Africa to establish this crop in summer season (Gupta et al., 2015). Pearl millet is also cultivated as a valuable fodder in limited areas in Australia and Latin America and as forage and cover crop (mulch) in Brazil (Calegari et al., 2014; França & Miyagi, 2012; Pacheco & Petter, 2011). The production areas in Asia and Africa, which together account for up to 84% of global millet production, are mainly characterized by high temperatures, low and erratic rainfall, poor soil fertility, and frequent occurrence of diseases and insect pests. It serves as a food crop for ~90 million people in these regions (Yadav & Rai, 2013). Pearl millet is also highly input responsive, and improved varieties can yield up to 4–5 t grain ha<sup>-1</sup> in certain environments. It is also reported that pearl millet has a high outcrossing rate of ~90% and show high diversity both at phenotypic and genotypic level (Satyavathi et al., 2013; Singh et al., 2013; Wilson, Burton, Zongo, & Dicko, 1990). Recently, a study conducted by Burgarella et al. (2018) provided evidence that wild-to-crop gene flow increased cultivated genetic diversity in pearl millet leading to diversity hotspots in western and eastern Sahel and adaptive introgression of 15 genomic regions.

Pearl millet is more nutritious than wheat (*Triticum aestivum* L.), rice (*Oryza sativa* L.), maize (*Zea mays* L.), and sorghum [*Sorghum bicolor* (L.) Moench] (Agte, Khot, Girigosavi, Paknlkar, & Chiplonkar, 1999; Muthamilarasan, Dhaka, Yadav, & Prasad, 2016; Vadez, Hash, Bidinger, & Kholova, 2012) and offers gluten-free grains with high content and better quality of protein, vitamins, antioxidants, essential micronutrients such as iron and zinc, and more balanced essential amino acid profile than maize or sorghum (Agte et al., 1999; Anitha, Govindaraj, & Kane-Potaka, 2019; Nambiar, Dhaduk, Sareen, Shahu, & Desai, 2011; Rai, Gowda, Reddy, & Sehgal, 2008). Because of its high nutritional value, it is also known as ‘nutrigrain’ or high-energy cereal. Pearl millet is also a useful cover crop because of its ability to accumulate high amount of N, P, K, Ca, and Mg in the dry matter (Boer et al., 2007).

Though pearl millet is better adapted to hot and dry conditions and infertile soils than other cereal crops, climate change will expose the crop to more adverse climatic conditions particularly more severe drought and heat stress. Drought is the

### Core Ideas

- Importance and use of wild *Pennisetum* species for pearl millet improvement.
- Stage-gate concept for better management of pre-breeding using wild species.
- Strategies to enhance use of wild species for germplasm enhancement in crop improvement.

biggest challenge for this crop in the African and Asian ecologies, while heat stress is more pronounced in northwestern India and some western African countries. Besides abiotic stresses, biotic stress such as blast caused by *Pyricularia grisea* (T.T. Hebert) M.E. Barr (teleomorph: *Magnaporthe grisea*), has become an important disease of pearl millet in this decade. It is, therefore, essential to increase the drought and heat tolerance of current varieties together with high levels of resistance to blast. These research endeavors will improve the cultivation of pearl millet in traditional and nontraditional areas and increase the production and productivity of this crop in the arid and semi-arid regions.

This article mainly focuses on the importance of CWRs in enriching the gene pool for abiotic and biotic stresses and agronomic and nutritional traits. Crop wild relatives are the reservoir of beneficial genes and alleles for tolerance to various abiotic stresses and resistance to biotic stresses (Dempewolf et al., 2017; Kilian et al., 2011; Sharma, 2017). Diverse alleles of traits of importance from CWRs can play significant role in enhancing the genetic gains in cultivated crops especially under climate change scenarios.

## 2 | ORIGIN, DISTRIBUTION, TAXONOMY, AND GENE POOL

### 2.1 | Origin, domestication, and geographic distribution

Based on geographical diversity and distribution, Harlan (1971) and Harlan, de Wet, and Stemler (1975) proposed a defused belt extending from western Sudan to Senegal as the center of origin of pearl millet. The wild progenitor of pearl millet, *P. glaucum* ssp. *monodii* (Maire) Br. (Brunken, 1977; Harlan, 1975) occurs in the Sahel zone in Africa (Brunken, 1977; Harlan, 1975). The distribution of cultivated pearl millet and wild *Pennisetum* species overlap in the Sahel, where intermediate morphologies and genotypes are documented (Burgarella et al., 2018). The evolutionary history of pearl millet is not yet clearly established. However, some authors suggest that pearl millet originated through

multiple domestications in several regions distributed throughout the Sahel from Mauritania to western Sudan (Harlan, 1975; Portères, 1976), while others proposed a single domestication (Oumar, Mariac, Pham, & Vigouroux, 2008). A study based on isozyme surveys of wild populations and domesticated pearl millet varieties suggested the origin of pearl millet domestication in southeast Mauritania and western Mali (Tostain, 1992, 1998), whereas Oumar et al. (2008) identified eastern Mali and western Niger as possible regions for the domestication of pearl millet. Recently, Burgarella et al. (2018) supported the origin of pearl millet in the region corresponding to the Taoudeni Basin in western Sahara ( $-6.61^{\circ}$  E,  $23.58^{\circ}$  N) and the onset of diffusion of pearl millet agriculture  $\sim 4,892$  yr ago supporting a Saharan cradle of pearl millet domestication. It is reported that drying of the then wetter Sahara led the plant communities including pearl millet to move south to the current distribution in the central Sahel  $\sim 3,200$  yr ago (Kröpelin et al., 2008). The study conducted by Burgarella et al. (2018) also provided the evidences that worldwide cultivated pearl millet varieties were derived from a common ancestor of wild populations found today in the central Sahel and the wild-to-crop gene flow during its agricultural diffusion increased cultivated genetic diversity leading to diversity hotspots in western and eastern Sahel outside the center of origin. These results also fit well with the recent archeological hypothesis wherein the oldest archaeobotanical evidence of 4,500-yr-old domesticated pearl millet was found in the lower Tilemsi Valley in north-eastern Mali (Manning, Pelling, Higham, Schwenniger, & Fuller, 2011).

It is reported that the domestication first produced early maturing cultivars (Tostain & Marchais, 1989). The early maturing forms of domesticated pearl millet were brought to eastern Africa  $\sim 3,000$  B.C. (Tostain, 1998; Tostain & Marchais, 1993) facilitated by their efficient adaptation to arid conditions (D'Andrea & Casey, 2002) and then to India (D'Andrea, Klee, & Casey, 2001; Khairwal et al., 2007). India is regarded as the secondary center of diversity (Brunken, de Wet, & Harlan, 1977).

Around 2,010 yr B.C., a further diffusion took place in the region near Lake Chad (on the Nigerian side; Klee, Zach, & Stika, 2004) in which photoperiod-sensitive varieties were selected. This led to the development of a secondary center of diversity in this region. These late-maturing lines were transported further into the Sudanian zone of southwest Africa from northern Nigeria to southern Senegal (Tostain & Marchais, 1993; Tostain, Riandey, & Marchais, 1987). These lines were adapted to the humid conditions in the southern Sudanian zone (D'Andrea & Casey, 2002; Tostain, 1998). About 1,000 yr B.C., pearl millet was transported toward the plateau of southern Africa via Uganda and to Namibia (Tostain, 1998; Tostain & Marchais, 1993).

The most recent introduction of this crop was in the United States and Brazil; records of the cultivation of this crop are available in the United States since the 1850s and in Brazil in the 1960s (National Institute of Plant Health Management, 2014).

The domestication process of pearl millet is associated with frequent morphological changes such as suppression of spikelet shedding, size reduction of bristles and bracts, increase in seed size, increase in spikelet pedicel length, loss of dormancy, decrease in the number of basal tillers, and increase in spikelet length (Poncet et al., 1998).

## 2.2 | Taxonomic classification and the gene pool

The genus *Pennisetum* Rich. belongs to the family Poaceae, the subfamily Panicoideae, and the tribe Paniceae. This genus is closely related to the genus *Cenchrus* L. (Bor, 1960; Stapf & Hubbards, 1934) and both are placed within the bristle clade in the tribe Paniceae along with  $\sim 23$  other genera (*Ixophorus* Schldl., *Paspalidium* Stapf, *Setaria* P.Beauv., and others) (Bess, Doust, & Kellogg, 2005; Doust & Kellogg, 2002). The characteristics, such as degree of fusion of the bristles, the presence of pedicellate spikelets, and type of bristles (flat or stiff), are commonly used to separate *Pennisetum* from *Cenchrus* (Clayton & Renvoize., 1982, 1986; Watson & Dallwitz, 1992); however, none of them can be effectively used to segregate the two genera (Webster, 1988). Kellogg, Aliscioni, Morrone, Pensiero, and Zuloaga (2009) also placed the genus *Odontelytrum*, harboring only a single species, *O. abyssinicum*, in this clade along with two genera: *Pennisetum* and *Cenchrus*. A study based on a combined nuclear, plastid, and morphological analysis proposed the unification of three genera *Pennisetum*, *Cenchrus*, and *Odontelytrum* (Chemisquy, Giussani, Scataglini, Kellogg, & Morrone, 2010). Similarly, based on the chromosomal and genomic characteristics along with phylogenetic relationships, Robert et al. (2011) favored the inclusion of *Cenchrus* species in the genus *Pennisetum*. Therefore, it has been proposed to reconsider the taxonomic position of the *Cenchrus* species and to rename them into genus *Pennisetum* as previously known (Robert et al., 2011).

The genus *Pennisetum* comprises  $\sim 80$ –140 species (Brunken, 1977) with different basic chromosome numbers ( $x = 5, 7, 8, \text{ or } 9$ ) (Jauhar, 1981), ploidy levels (diploid to octoploid), reproductive behavior (sexual or apomictic), and life cycle (annual, biennial, or perennial) (Martel, De Nay, Siljak-Yakovlev, Brown, & Sarr, 1997). Phylogenetic analysis suggested that the chromosome complement in *Pennisetum* has evolved from a basic chromosome number of  $x = 9$  with a short length (Martel et al., 2004). Species with basic

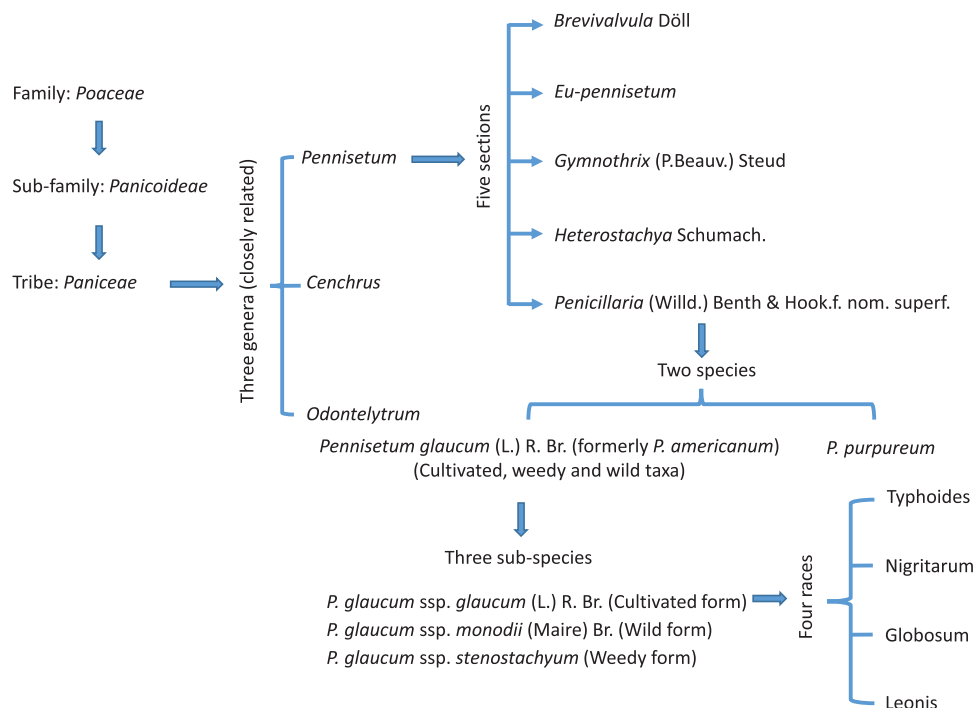


FIGURE 1 Taxonomic classification of the genus *Pennisetum*

chromosome numbers of  $x = 5, 7,$  and  $8$  appear in the most recent divergent clades, suggesting that the genome structure in *Pennisetum* may have evolved toward a reduced chromosome number and an increased chromosome size (Martel et al., 2004), which is consistent with the chromosome evolutionary trend generally observed in grasses (Martel et al., 2004).

Based on morphological characteristics, genus *Pennisetum* is classified into five sections (Stapf & Hubbard, 1934; Table 1; Figure 1): *Brevivalvula* Döll (pan-tropical), *Eu-pennisetum* (tropical and subtropical Africa and Asia), *Gymnothrix* (P.Beauv.) Steud (pantropical), *Heterostachya* Schumach. (northeastern Africa), and *Penicillaria* (Willd.) Benth & Hook.f. nom. superf. (tropical Africa and India), each having a variable number of species with variable basic chromosome number (Table 1). The annual diploid cultivated pearl millet, *P. glaucum* (L.) R. Br. [former *P. americanum* (L.) Leeke; syn. *P. glaucum* ssp. *glaucum*] along with its diploid wild species, *P. glaucum* ssp. *monodii* (Maire) Br., the diploid weedy species, *P. glaucum* ssp. *stenostachyum* (Klotzsch ex Müll. Berol.) Brunken (all species with  $2n = 2x = 14$ ) and the reproductively isolated perennial tetraploid *P. purpureum* Schumach ( $2n = 4x = 28$ ) are placed in the section *Penicillaria* (Martel et al., 2004) (Figure 1).

Based on the cross-compatibility relationship between cultivated pearl millet and CWRs, these species were classified into primary (GP1), secondary (GP2), and tertiary (GP3) gene pools following Harlan and de Wet (1971) (Supplemental Table S1). The GP1 consists of the following:

1. Domesticated diploid species, *P. glaucum* ssp. *glaucum* ( $2n = 2x = 14$  with AA genome);
2. Wild progenitor, *P. glaucum* ssp. *monodii* with two ecotypes ( $2n = 2x = 14$  with AA genome):
  - (i) *Pennisetum violaceum* (Lam.). L. Rich. (also known as *P. glaucum* ssp. *monodii* forma *violaceum*), and
  - (ii) *Pennisetum mollissimum* Hochst. (also known as *P. glaucum* ssp. *monodii* forma *mollissimum* Hochst.)]
3. Weedy forms, *shibras* [= *P. glaucum* ssp. *stenostachyum* Kloyzesh ex. Müll. Berol. Brunken;  $2n = 2x = 14$  with AA genome].

Members of GP1 easily cross under sympatric conditions and form fertile hybrids with normal chromosome pairing (Harlan & de Wet, 1971) and thus have high possibility of successfully introgressing genes from CWRs into cultivated pearl millet.

The GP2 includes an allotetraploid rhizomatous perennial species, *P. purpureum*, also known as Napier grass or elephant grass ( $2n = 4x = 28$  with A'A'BB genome), and the apomictic and octaploid species *P. squamulatum* Fresen. ( $2n = 8x = 56$ ) (Kaushal et al., 2007). *Pennisetum purpureum* and *P. squamulatum* can be easily crossed with cultivated pearl millet but their hybrids are highly sterile.

The GP3 includes the remaining species that are cross-incompatible with cultivated pearl millet. In GP3, *P. schweinfurthii* (= *P. tetrastachyum*) Pilg. is the only *Pennisetum* species to have  $2n = 2x = 14$  large chromosomes with an annual growth habit (Martel et al., 2004) but its chromosomes

TABLE 1 List of species in genus *Pennisetum*

Section		Species	Chromosome no. (2n)	Basic chromosome no. (x)	Geographical distribution
<i>Penicillaria</i>	1	<i>P. glaucum</i> (L.) R. Br.			
	1.1	<i>P. glaucum</i> ssp. <i>glaucum</i> (L.) R. Br. (Pearl millet)	14 (2x)	7	Semi-arid tropics of Africa and India
	1.2	<i>P. glaucum</i> ssp. <i>monodii</i> (Maire) Brunken	14 (2x)	7	Sahel in western Africa
	a)	<i>P. violaceum</i> (Lam.) L. Rich. (ecotype)	14 (2x)	7	Africa
	b)	<i>P. mollissimum</i> Hochst. (ecotype)	14 (2x)	7	West Africa
	1.3	<i>P. glaucum</i> ssp. <i>stenostachyum</i> (Klotzsch ex Müll. Berol.) Brunken	14 (2x)	7	Weedy form
	2	<i>P. purpureum</i> Schumach.	28 (4x)	7	Wet tropics of the world
<i>Heterostachya</i>	3	<i>P. orientale</i> L.C. Rich.	36 (4x)	9	White fountaingrass, Oriental Pennisetum–North Africa, Middle East, Central Asia, Indian Subcontinent
	4	<i>P. schweinfurthii</i> (= <i>P. tetrastachyum</i> ) Pilg.	14 (2x)	7	Ethiopia, Sudan
	5	<i>P. squamulatum</i> Fresen.	54 (6x), 56 (8x)	9, 7	Africa
<i>Brevivalvula</i>	6	<i>P. hordeoides</i> Steud.	18 (2x), 36 (4x), 54 (6x)	9	Africa, Asia-tropical
	7	<i>P. pedicellatum</i> Trin.	54 (6x)	9	–
	7.1	<i>P. pedicellatum</i> ssp. <i>pedicellatum</i> Trin.	36, 45, 52, 54	– <sup>a</sup>	Ethiopia
	7.2	<i>P. pedicellatum</i> ssp. <i>unispiculum</i> Brunken	35+1B, 36, 45, 54	–	–
	8	<i>P. polystachion</i> (L.) Schult.	54 (6x), 36 (4x), 63 (7x)	9	Tropical Africa, Australia, Sri Lanka
	8.1	<i>P. polystachion</i> ssp. <i>polystachion</i> (L.) Schult.	18 (2x) - 54 (6x)	9	Africa, southern Asia from Arabia to Vietnam, Indian Ocean islands
	8.2	<i>P. polystachion</i> ssp. <i>atrichum</i> Stapf & C.E. Hubb.	36 (4x)	9	Marquesas
<i>Eu-Pennisetum</i>	9	<i>P. clandestinum</i> Hochst. ex Chiov.	36 (4x)	9	Central and Eastern Africa
	10	<i>P. flaccidum</i> Griseb.	18 (2x), 36 (4x)	9	Himalayas, Central Asia, China, Mongolia
	11	<i>P. foermerianum</i> Leeke.	–	–	Namibia
	12	<i>P. setaceum</i> (Forssk.) Chiov.	27 (3x), 54 (6x)	9	Northern Africa, southwestern Asia; naturalized in Australia, New Zealand, scattered places in Europe and Americas
	13	<i>P. sieberianum</i> (Schltdl.) Stapf & C.E.Hubb.	–	–	Africa
	14	<i>P. villosum</i> R.Br. ex Fresen.	36 (4x), 45 (5x), 54 (6x)	9	Sahara, Sahel
<i>Gymnothrix</i>	15	<i>P. alopecuroides</i> (L.) Spreng	18 (2x)	9	Australia, East and Southeast Asia
	16	<i>P. basedowii</i> Summerh. & C.E. Hubb.	54 (6x)	9	Australia
	17	<i>P. chilense</i> (Desv.) Hack.	–	–	Chile, Argentina, Peru, Bolivia
	18	<i>P. frutescens</i> Leeke	63	–	Paraguay, Argentina
	19	<i>P. hohenackeri</i> Hochst. ex Steud.	18 (2x)	9	Kenya, Tanzania, Uganda, Madagascar, India, Nepal, Pakistan
	20	<i>P. latifolium</i> Spreng.	36 (4x)	9	South America from Colombia to Uruguay

(Continues)



TABLE 1 (Continued)

Section	Species	Chromosome no. (2n)	Basic chromosome no. (x)	Geographical distribution
	21 <i>P. macrourum</i> Trin.	36 (4x), 54 (6x)	9	African feather grass, bedding grass, waterside-reed–Africa, Yemen, Saudi Arabia
	22 <i>P. massaicum</i> Stapf	32 (4x)	8	Somalia, Kenya, Tanzania, Zimbabwe
	23 <i>P. mezianum</i> Leeke	32 (4x)	8	Ethiopia, Sudan, South Sudan, Kenya, Uganda, Tanzania, Namibia, Limpopo
	24 <i>P. montanum</i> (Griseb.) Hack.	32 (4x)	8	Peru, Bolivia, Argentina
	25 <i>P. nervosum</i> (Nees) Trin.	36 (4x)	9	Bent spike fountain grass–South America; naturalized in Belize, Nicaragua, Mexico, California, Texas
	26 <i>P. ramosum</i> (Hochst.) Schweinf	10 (2x)	5	Central + eastern Africa
	27 <i>P. sphacelatum</i> (Nees) T. Durand & Schinz	18 (2x)	9	Africa, Comoros
	28 <i>P. thunbergii</i> Kunth	18 (2x)	9	Africa, Yemen
	29 <i>P. trachyphyllum</i> Pilg.	–	–	Central Africa
	30 <i>P. tristachyum</i> (Kunth) Spreng.	–	–	South America
	31 <i>P. unisetum</i> (Nees) Benth.	18	9	Natal grass, silky grass–Africa, Yemen, Saudi Arabia
Unknown	32 <i>P. advena</i> Wipff & Veldkamp	–	–	North America
	33 <i>P. annuum</i> Mez	–	–	Peru
	34 <i>P. articulare</i> Trin.	–	–	Marquesas
	35 <i>P. bambusiforme</i> (E. Fourn.) B.D. Jacks.	36 (4x)	9	America
	36 <i>P. beckeroides</i> Leeke	–	–	Ethiopia
	37 <i>P. caffrum</i> (Bory) Leeke.	–	–	Madagascar, Réunion
	38 <i>P. centrasiaticum</i> Tzvelev	36 (4x)	9	Asia-temperate
	39 <i>P. ciliare</i> L. link (syn. <i>Cenchrus ciliaris</i> L.)	32, 36, 40, 54, 43, 48, 63, 90	9	Africa, West Asia, India
	40 <i>P. complanatum</i> (Nees) Hemsl.	–	–	Nicaraguan fountain grass–Veracruz, Central America
	41 <i>P. crinitum</i> (Kunth) Spreng.	20, 40	–	Mexico
	42 <i>P. distachyum</i> (E. Fourn.) Rupr. ex Chase	36 (4x)	9	Mexico, Costa Rica, Guatemala
	43 <i>P. divisum</i> (Forssk. ex J.F. Gmel.) Henrard	36 (4x)	9	Deserts from Mauritania to western India
	44 <i>P. domingense</i> (Spreng.) Spreng.	–	–	Cuba, Hispaniola
	45 <i>P. durum</i> Beal	–	–	Mexico
	46 <i>P. glaucifolium</i> Hochst. ex A. Rich.	–	–	Eritrea, Ethiopia
	47 <i>P. gracilescens</i> Hochst.	–	–	Eritrea, Ethiopia, Sudan
	48 <i>P. henryanum</i> F. Br.	–	–	Marquesas
	49 <i>P. humile</i> Hochst. ex A.Rich.	–	–	Ethiopia
	50 <i>P. intectum</i> Chase	–	–	Peru, Ecuador
	51 <i>P. lanatum</i> Klotzsch	18	–	Afghanistan, northern India, Pakistan, Tajikistan, Nepal, Tibet
	52 <i>P. laxius</i> (Clayton) Clayton	–	–	Sahel in Africa
	53 <i>P. ledermannii</i> Mez	–	–	Cameroon
	54 <i>P. longissimum</i> S.L. Chen & Y.X. Jin	54 (6x)	9	China
	55 <i>P. longistylum</i> Hochst. ex A. Rich.	54 (6x)	9	Eritrea, Ethiopia
	56 <i>P. macrostachyum</i> (Borongn.) Trin.	54 (6x)	9	Pacific fountain grass–Asia tropical, Pacific

(Continues)

TABLE 1 (Continued)

Section	Species	Chromosome no. (2n)	Basic chromosome no. (x)	Geographical distribution
	57 <i>P. mildbraedii</i> Mez	–	–	Rwanda, Zaire, Uganda
	58 <i>P. monostigma</i> Pilg.	18 (2x)	9	Sierra Leone, Nigeria, Cameroon, islands in Gulf of Guinea
	59 <i>P. nodiflorum</i> Franch	–	–	Central Africa
	60 <i>P. nubicum</i> (Hochst.) K.Schum. ex Engl.	–	–	Eritrea, Ethiopia, Sudan, Saudi Arabia
	61 <i>P. occidentale</i> Chase	–	–	Venezuela, Colombia, Ecuador, Peru
	62 <i>P. pauperum</i> Steud.	–	–	Ecuador incl Galápagos
	63 <i>P. peruvianum</i> Trin.	36 (4x)	9	Venezuela, Colombia, Ecuador, Peru
	64 <i>P. petiolare</i> (Hochst.) Chiov.	–	–	Petioled fountain grass–Eritrea, Ethiopia, Sudan
	65 <i>P. pirottae</i> Chiov.	–	–	Eritrea, Ethiopia, Sudan
	66 <i>P. procerum</i> (Stapf) Clayton	–	–	Uganda, Kenya
	67 <i>P. prolificum</i> Chase	–	–	Southern Mexico
	68 <i>P. pseudotriticoides</i> A. Camus	18	–	Madagascar
	69 <i>P. pumilum</i> Hack. ex Engl.	–	–	Ethiopia
	70 <i>P. qianningense</i> S.L. Zhong	36 (4x)	9	Sichuan, Yunnan
	71 <i>P. rigidum</i> (Griseb.) Hack.	–	–	Northern Argentina
	72 <i>P. riparium</i> Hochst. ex A. Rich.	–	–	East Africa
	73 <i>P. rupestre</i> Chase	–	–	Colombia, Peru
	74 <i>P. sagittatum</i> Henrard	–	–	Peru, Bolivia
	75 <i>P. setigerum</i> (Syn. <i>Cenchrus setiger</i> ) (Vahl) Wipff	34 (2x), 36, 46, 54	–	North-east Africa, India
	76 <i>P. shaanxiense</i> S.L. Chen & Y.X. Jin	–	–	China
	77 <i>P. sichuanense</i> S.L. Chen & Y.X. Jin	–	–	Sichuan, Yunnan
	78 <i>P. stramineum</i> Peter	–	–	Eritrea, Ethiopia, Kenya, Tanzania, Yemen, Saudi Arabia
	79 <i>P. tempisqueense</i> R.W. Pohl	72 (8x)	9	Costa Rica
	80 <i>P. thulinii</i> S.M. Phillips	–	–	Ethiopia
	81 <i>P. trisetum</i> Leeke	36 (4x)	9	Central Africa
	82 <i>P. uliginosum</i> Hack. ex Engl.	–	–	Ethiopia
	83 <i>P. weberbaueri</i> Mez	–	–	Ecuador, Bolivia, Peru
	84 <i>P. yemense</i> Deflers	–	–	Yemen, Saudi Arabia, Eritrea

Sources: <http://www.theplantlist.org>; <http://ccdb.tau.ac.il/Angiosperms/Poaceae/Pennisetum>; <https://en.wikipedia.org/wiki/Pennisetum>.

<sup>a</sup>–, unknown or information not available.

are nonhomologous (Hanna & Dujardin, 1986) with different genomic localizations of rDNA probes (Martel, Ricroch, & Sarr, 1996). There are strong reproduction barriers between the members of GP3 and GP1 or GP2, and gene transfer is only possible by radical manipulations involving in vitro techniques or by using complex hybrid bridges. Tertiary gene pool species with a basic chromosome number of 9 ( $x = 9$ ) are more likely to cross with pearl millet than those with  $x = 5$  [*P. ramosum* (Hochst.) Schweinf] or  $x = 8$  (*P. meizianum* Leeke) (Dujardin & Hanna, 1989a).

### 3 | CWR OF PEARL MILLET: CURRENT STATUS IN GENE BANKS

For the genetic improvement of cultivated pearl millet, about ~4,900 accessions of 56 wild *Pennisetum* species are conserved in 52 genebanks in 38 countries ([http://www.fao.org/views-archive/germplasm\\_report.jsp](http://www.fao.org/views-archive/germplasm_report.jsp); <http://genebank.icrisat.org>) (Figure 2). In total, ~91% of this wild germplasm collection is conserved in 10 genebanks in eight countries. (Table 2).

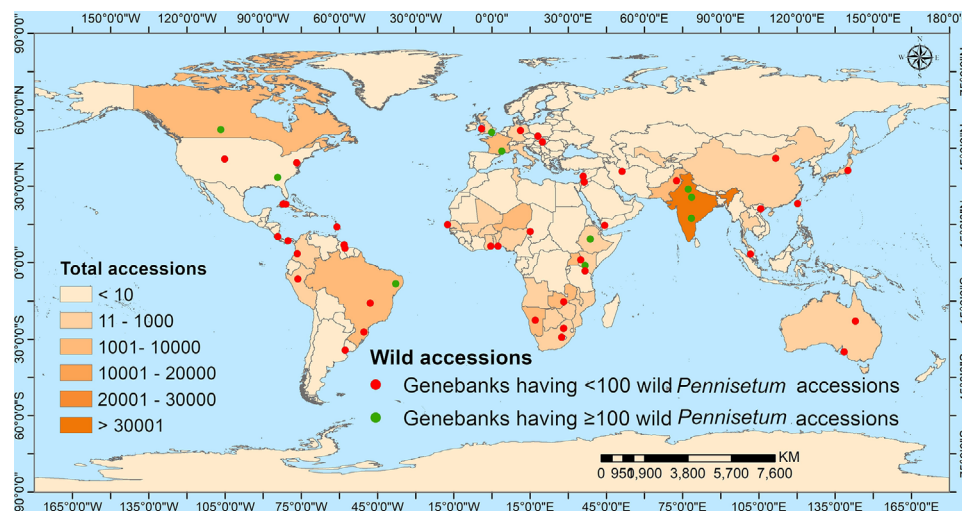


FIGURE 2 Genebanks conserving wild *Pennisetum* accessions across the globe

The highest number of wild *Pennisetum* accessions (963 accessions) are conserved at the Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USDA–ARS, United States, followed by 798 wild *Pennisetum* accessions at the Laboratoire des Ressources Génétiques et Amélioration des Plantes Tropicales, ORSTOM, France, and 794 wild *Pennisetum* accessions at the RS Paroda genebank at ICRISAT, Patancheru, India (Table 2). The RS Paroda genebank holds the world's largest collection of pearl millet germplasm (24,373 accessions) consisting of landraces, obsolete varieties, breeding lines, cultivars, and CWRs from 51 countries ([genebank.icrisat.org](http://genebank.icrisat.org)) (Figure 3). In addition to *Pennisetum*, 3,758 accessions of 24 *Cenchrus* species are also conserved in 51 genebanks in 33 countries ([http://www.fao.org/wiews-archive/germplasm\\_report.jsp](http://www.fao.org/wiews-archive/germplasm_report.jsp)). These plant genetic resources provide abundant natural genetic variations for use in pearl millet improvement programs.

#### 4 | IMPORTANCE AND USE OF WILD *PENNISETUM* SPECIES IN PEARL MILLET IMPROVEMENT

High genetic differentiation between cultivated pearl millet and wild *Pennisetum* species was observed using microsatellite markers, which revealed that only 74% of the genetic diversity of wild relatives is found in the domesticated groups (Oumar et al., 2008), suggesting that domestication has led to a decrease in genetic diversity. Mariac et al. (2006) also observed significantly lower number of alleles and lower gene diversity in cultivated pearl millet accessions than wild accessions. These studies suggest that wild populations could serve as a potent source for capturing new allelic variants associated

with climate resilient traits to broaden the genetic base of cultivated pearl millet. It is also evident from these studies that domestication and selection processes have led to the significant loss of diversity in cultivated accessions (Figure 4).

For continuous improvement in the cultivar development, novel and diverse sources of variations are needed to introgress high frequency of useful alleles and genes. The wild *Pennisetum* species are a treasure trove of novel and useful alleles for important biotic and abiotic stresses, forage yield, and quality-related traits and also provide source of cytoplasmic male sterility (CMS) (Table 3). The CWRs have been exploited in the past mainly for the introgression of pest and disease resistance in different crops such as rice, wheat, cotton, upland (*Gossypium hirsutum* L.), potato (*Solanum tuberosum* L.), groundnut (*Arachis hypogaea* L.), etc. (Anjum et al., 2015; Brar & Kush, 1997; Chandel et al., 2015; Hoisington et al., 1999; Kaneko & Bang, 2014; Khush et al., 1990; Nazeer et al., 2014; Sharma, Pandey, Sudini, Upadhyaya, & Varshney, 2017; Simpson & Starr, 2001; Simpson, Starr, Church, Burow, & Paterson, 2003; Suszkiw, 2005; Tarwacka, Polkowska-Kowalczyk, Bozena, Jadwiga, & Bernard, 2013), and the researchers continue to explore novel alleles in extended gene pools for new variations.

In pearl millet, the most important biotic stress factors that negatively influence its productivity are the diseases such as downy mildew (*Sclerospora graminicola*), blast (*Pyricularia grisea*; teleomorph: *Magnaporthe grisea*), rust (*Puccinia substriata* var. *indica*), ergot (*Claviceps fusiformis*), and smut (*Moesziomyces penicillariae*). In western and central Africa, south of the Sahara, *Striga hermonthica* (Del.) Benth. is the greatest biotic stress and persistent threat to pearl millet cultivation (Wilson, Hess, Hanna, Kumar, & Gupta, 2004). In India, main research focus of pearl millet breeding programs is on broad-spectrum downy mildew resistance, blast resistance,



TABLE 2 Major genebank holdings of wild *Pennisetum* germplasm across globe

Country	Institute	No. of wild <i>Pennisetum</i> accessions
Brazil	Empresa Pernambucana de Pesquisa Agropecuária [IPA (BRA043)]	500
Canada	Plant Gene Resources of Canada, Saskatoon Research Centre, Agriculture and Agri-Food Canada [PGRC (CAN004)]	259
Ethiopia	International Livestock Research Institute [ILRI-Ethiopia (ETH013)]	178
France	Laboratoire des Ressources Génétiques et Amélioration des Plantes Tropicales, ORSTOM [ORSTOM-MONTP (FRA202)]	798
India	International Crop Research Institute for the Semi-Arid Tropics [ICRISAT (IND002)]	794
India	National Bureau of Plant Genetic Resources [NBPGR (IND001)]	226
India	Indian Grassland and Fodder Research Institute [IGFRI (IND027)]	146
Kenya	National Genebank of Kenya, Crop Plant Genetic Resources Centre–Muguga [KARI-NGBK (KEN015)]	327
United Kingdom	Millennium Seed Bank Project, Seed Conservation Department, Royal Botanic Gardens, Kew, Wakehurst Place [RBG (GBR004)]	246
United States	Plant Genetic Resources Conservation Unit, Southern Regional Plant Introduction Station, University of Georgia, USDA–ARS [S9 (USA016)]	963

and rust. Ergot and smut are sporadic diseases, and insects are considered minor pests in India. Therefore, no great efforts are made to breed for ergot, smut, and insect-pest tolerance in pearl millet.

Wild relatives of pearl millet have been explored to identify resistance to pearl millet downy mildew (Table 3). Singh and Navi (2000) investigated 529 accessions of wild *Pennisetum* species [*P. violaceum*, *P. mollissimum*, *P. purpureum*, *P. pedicellatum* Trin., and *P. polystachyon* (L.) Schult.] in greenhouse and field-disease nurseries for downy mildew resistance, of which, 223 accessions were found free from this disease. High level of downy mildew resistance in *P. schweinfurthii*, coupled with resistance to rust, can be used in disease-resistance breeding programs because of the cross-compatibility of this species with pearl millet. In an effort to introgress downy mildew from CWRs, two wild *Pennisetum* species, *P. pedicellatum*, and *P. polystachion* were crossed with cultivated pearl millet but no hybrid could be formed (Dujardin & Hanna, 1989a).

Once considered a disease of minor importance, blast disease has become a major biotic constraint for pearl millet in the last 10–12 yr in India. Breeding for blast resistance was not as high a priority as breeding for downy mildew resistance, but strict monitoring was a regular feature of the Indian national testing system to ensure that no susceptible varieties were released for cultivation. Indeed, because of the high and widespread occurrence of blast disease in India, the focus of pearl millet research has been shifted from downy mildew to blast research. *Magnaporthe grisea*, which causes pearl millet blast, is a highly variable pathogen, and the rapid change in the pathogenicity of this fungus has been cited as the main cause for the breakdown of resistance genes in rice as a result of its race specificity (Suh et al., 2009). Pathogenic variation in the *M. grisea* isolates collected from pearl millet has been reported (Sharma et al., 2013). Therefore, for the management of pearl millet blast through host-plant resistance, it is essential to identify resistance sources against different pathotypes of the pathogen. Resistance to blast was identified in *P. glaucum* ssp. *monodii* accession in the United States. The blast resistance in this accession was found to be conferred by three independent dominant genes (Hanna & Wells 1989), although Tift 85DB, with resistance derived from *P. glaucum* ssp. *monodii*, was shown to have a single resistance gene (Wilson, Wells, & Burton, 1989). This resistance gene was effective against several *Pyricularia* isolates tested until the late 1990s in the United States (Morgan, Wilson, Hanna, & Ozias-Akins, 1998). Recently, sources of blast resistance have also been identified from *P. violaceum* (= *monodii* Maire) against *M. grisea* isolates in India. Sharma, Sharma, and Gate (2020) screened 305 accessions of *P. violaceum* under greenhouse conditions against five pathotype-isolates (Pg 45, Pg 53, Pg 56, Pg 118, and Pg 119) of *M. grisea* to identify resistance sources effective against the pathogen populations prevalent

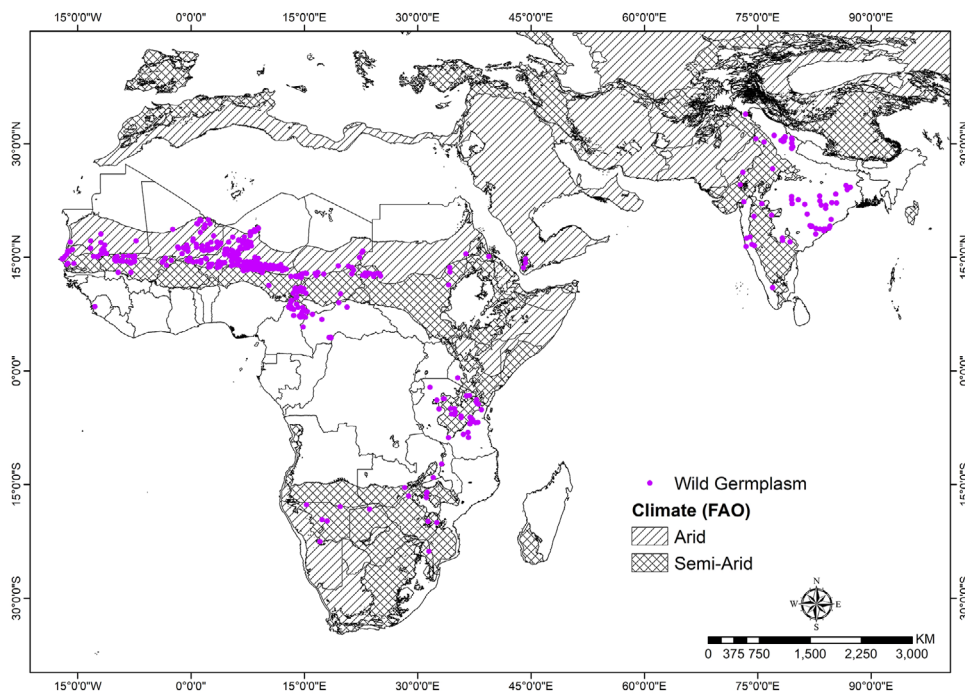


FIGURE 3 Geographic distribution of wild *Pennisetum* species conserved in ICRISAT gene bank, Patancheru, India

**Pre-breeding: A link between genebanks and breeding programs**

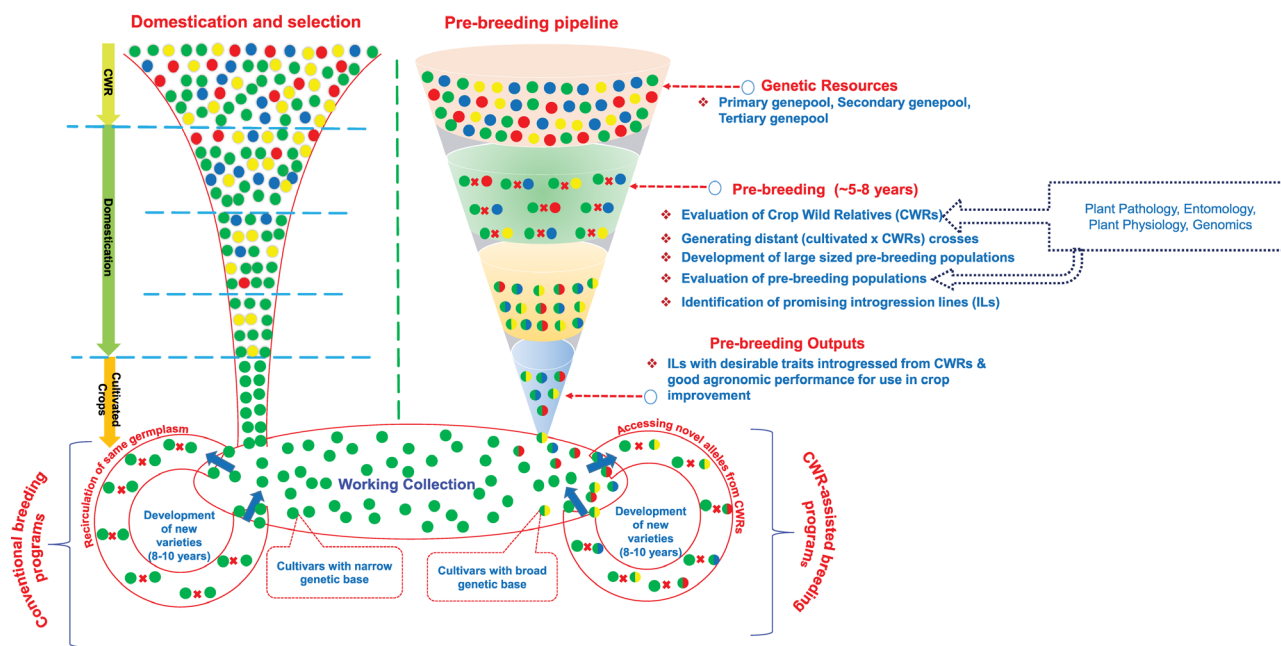


FIGURE 4 Prebreeding: a link between genebanks and breeding programs (modified from Sharma, 2017)

in India. Seventeen accessions resistant (score  $\leq 3.0$ ) to all the five pathotypes were identified as potential sources of blast resistance (Table 3).

Rust is another important leaf disease that reduces seed yield in the hybrid seed production fields and adversely affects biomass and quality of forage in pearl millet (Monson, Hanna, & Gaines, 1986; Wilson, Hanna, & Gascho, 1996). Rust infec-

tion has been reported to cause yield losses of up to 72% in pearl millet in the southeastern United States (Wilson et al., 1996), and its natural occurrence in severe form has been reported from Brazil and India as well (de Carvalho et al., 2006; Monson et al., 1986; Singh & King, 1991). The original source of rust resistance that was used to develop pearl millet varieties adapted to the southern United States was derived

TABLE 3 Wild *Pennisetum* species as novel sources of variations for important traits

Trait	Species	Promising accessions	References
A. Disease resistance sources			
Blast	<i>P. hordeoides</i> Steud.	–	Wilson & Hanna, 1992
	<i>P. pedicellatum</i> Trin.	–	Wilson & Hanna, 1992; Rai, Appa Rao, & Reddy, 1997; Singh et al., 1997; Singh & Navi, 2000; Shivhare & Lata, 2017
	<i>P. polystachion</i> ssp. <i>atrichum</i> Stapf & C.E. Hubb.	–	Wilson & Hanna, 1992
	<i>P. setosum</i> (Sw.) Rich.	–	Wilson & Hanna, 1992
	<i>P. violaceum</i> (Lam.) Rich.	IP 21525, 21531, 21536, 21540, 21594, 21610, 21640, 21706, 21711, 21716, 21719, 21720, 21721, 21724, 21987, 21988, 22160	Sharma et al., 2020
Downey mildew	<i>P. glaucum</i> ssp. <i>monodii</i> (Maire) Brunken	PS 202	Wilson et al., 2004
	<i>P. pedicellatum</i> Trin.	–	Rai et al., 1997
	<i>P. polystachion</i> L. Schult.	IPW 407	Singh et al., 1997; Singh & Navi, 2000; Shivhare & Lata, 2017
	<i>P. schweinfurthii</i> (= <i>P. tetrastachyum</i> ) Pilg.	IPW 151, 152, 153, 155	Singh & Navi, 2000
Rust	<i>P. glaucum</i> ssp. <i>monodii</i> (Maire) Brunken	–	Hammons, 1970
	<i>P. pedicellatum</i> Trin.	–	Wilson & Hanna, 1992; Singh et al., 1997; Singh & Navi, 2000; Shivhare & Lata, 2017
	<i>P. polystachion</i> (L.) Schult.	–	Wilson & Hanna, 1992; Rao, Reddy, & Bramel, 2003; Singh & Navi, 2000; Singh et al., 1997; Shivhare & Lata, 2017
	<i>P. subangustum</i> (Schumach.) Stapf & C. E. Hubbard	–	Wilson & Hanna, 1992; Singh et al., 1997; Shivhare & Lata, 2017
	<i>P. violaceum</i> (Lam.) Rich.	IP 21629, 21645, 21658, 21660, 21662, 21711, 21974, 21975, 22038	Hanna et al., 1987; Sharma et al., 2020
Leaf spot	<i>P. glaucum</i> ssp. <i>monodii</i> (Maire) Brunken	–	Hammons, 1970
B. <i>Striga</i> resistance			
<i>Striga</i>	<i>P. glaucum</i> subsp. <i>monodii</i> (Maire) Brunken	PS 64, 132, 190, 202, 208, 212, 287, 427, 428, 459, 549, 555, 622, 637, 639, 727, 755	Wilson et al., 2000, 2004
	<i>P. glaucum</i> ssp. <i>stenostachyum</i> (Klotzsch ex Müll. Berol.) Brunken	–	Wilson et al., 2000, 2004
	<i>P. hordeoides</i> Steud.	–	Koulengar, 1995; Ngarossal & Warou, 1993; Sy, 1994
	<i>P. pedicellatum</i> Trin.	–	Koulengar, 1995; Ngarossal & Warou, 1993; Sy, 1994

(Continues)

TABLE 3 (Continued)

Trait	Species	Promising accessions	References
C. Abiotic stress tolerance			
Salinity	<i>P. clandestinum</i> Hochst. Ex Chiov.	–	Muscolo et al., 2003, 2013
	<i>P. purpureum</i> Schumach.	–	Muscolo et al., 2003, 2013
Drought	<i>P. ciliare</i> L. Mant.	–	Duke, 1983
	<i>P. mezianum</i> Leeke.	–	Rai et al., 1997
	<i>P. orientale</i> L.C. Rich.	–	Dujardin & Hanna, 1987; Rai et al., 1997
Freezing and winter hardiness	<i>P. flaccidum</i> Griseb.	–	Stair, Dahmer, Bashaw, & Hussey, 1998; Muscolo et al., 2003
	<i>P. orientale</i> L. C. Rich.	–	Dujardin & Hanna, 1987; Stair et al., 1998; Muscolo et al., 2003
D. Male sterility (MS) and fertility restoration sources			
Cytoplasmic diversity	<i>P. schweinfurthii</i>		Hanna & Dujardin, 1986
MS source A <sub>4</sub>	<i>P. glaucum</i> subsp. <i>monodii</i> (Maire) Brunken	–	Hanna, 1989; Upadhyaya et al., 2007
MS source A <sub>4</sub> and Av	<i>P. violaceum</i> (Lam.) Rich.	–	Hanna, 1989; Marchais & Perne's, 1985
Fertility restoration	<i>P. purpureum</i> Schumach.	–	Hanna, 1990; Rai et al., 1997; Wilson & Hanna, 1992; Jauhar & Hanna, 1998
E. Fodder/Forage characters			
Forage yield, quality, and related traits	<i>P. cenchroides</i> Rich.	–	Marshall, Lewis, & Ostendorf, 2012
	<i>P. hordeoides</i> Steud.	–	Schmelzer, 1997
	<i>P. pedicellatum</i> Trin.	–	Schmelzer, 1997
	<i>P. purpureum</i> Schum.	–	Schmelzer, 1997; Robert et al., 2011
	<i>P. setosum</i> (Sw.) Rich.	–	Schmelzer, 1997
F. Ornamentals			
Ornamentals	<i>P. alopecuroides</i> (L.) Spreng.	–	Robert et al., 2011
	<i>P. flassidum</i> Griseb.	–	Robert et al., 2011
	<i>P. orientale</i> L.C. Rich.	–	Robert et al., 2011
	<i>P. setaceum</i> (Forssk.) Chiov.	–	Robert et al., 2011
G. Apomictic gene [apospory-specific genomic region (ASGR)]			
Apomixis	<i>P. ciliare</i> L. Mant. (= <i>Cenchrus ciliaris</i> L. Mant.)	–	Goel et al., 2006
	<i>P. orientale</i> L.C. Rich.	–	Hanna & Dujardin, 1982
	<i>P. setaceum</i> (Forssk.) Chiov.	–	Robert et al., 2011
	<i>P. squamulatum</i> Fresen.	–	Dujardin & Hanna, 1983b, 1985a, 1985b, 1989b; Rai et al., 1997; Goel et al., 2006; Robert et al., 2011

(Continues)

TABLE 3 (Continued)

Trait	Species	Promising accessions	References
H. Other agricultural related uses			
Growth rate and yield	<i>P. glaucum</i> subsp. <i>monodii</i> (Maire) Brunken	–	Bramel-Cox, Andrews, & Frey, 1986; Hanna, Wells, Burton, Hill, & Monson, 1988
Large seed size	<i>P. schweinfurthii</i> Pilg.	–	Serba et al., 2017
Soil bioengineering approach	<i>P. pedicellatum</i> Trin. (PPd)	–	Schmelzer et al., 1997; Ettbeb et al., 2020
	<i>P. polystachion</i> (L.) Schult. (PPL)	–	Ettbeb et al., 2020

from *P. glaucum* ssp. *monodii* from Senegal (Hanna, Wells, & Burton, 1985). This resistance gene was designated as *Rr1* and has been used to develop the parental lines Tift 85DB and Tift 65 (Burton & Wilson, 1995; Hanna, Wells, & Burton, 1987). Transfer of this gene into the inbred parents improved the rust resistance of hybrid cultivars; however, it turned out that this gene was unstable and lost its effectiveness against new races of the rust pathogen in the United States (Wilson, 1993). In India also, sources of resistance against rust have been identified from *P. violaceum*. The 305 accessions of *P. violaceum*, which were screened for blast resistance, were also screened for rust resistance (Sharma et al., 2020). Single-plant selections from nine accessions (IP no. 21629, 21645, 21658, 21660, 21662, 21711, 21974, 21975, and 22038) were found rust free after four generations of pedigree selection and subsequent screening.

There are not many reports on the identification and use of ergot and smut resistance from the wild relatives of pearl millet. Five accessions of *P. schweinfurthii* were screened against smut and ergot. All five accessions developed ergot (10–25%) and smut (5–15%) with artificial inoculation indicating moderate resistance in *P. schweinfurthii* to these diseases. Partial quantitative resistance to the parasitic weed *S. hermonthica* was reported in *P. glaucum* ssp. *monodii*, (Wilson, Hess, & Hanna, 2000). Four accessions (PS 202, PS 637, PS 639, and PS 727) of *P. glaucum* ssp. *monodii*, which have been shown to be resistant to *S. hermonthica* (Table 3), are probably useful sources of striga resistance for improving cultivated pearl millet in western Africa. One of these accessions, PS 202 also expressed resistance to downy mildew (Wilson et al., 2004).

Besides improving disease resistance, wild *Pennisetum* species, such as *P. purpureum*, is the potential donor for improving forage yield and quality, stalk strength, and providing restorer genes of the A<sub>1</sub> CMS (the first and currently most widely used CMS source) system (Jauhar & Hanna, 1998). *Pennisetum purpureum* thrives well on uncultivated lands with low water and low nutrient requirements. It has a high forage potential and is primarily used for grazing in dairy production in the tropics. Interspecific hybridiza-

tion between cultivated pearl millet and *P. purpureum* has led to the development of forage hybrids with high biomass and better quality (Hanna, Gaines, Gonzalez, & Monson, 1984; Jauhar & Hanna, 1998; Kannan, Valencia, & Altpeter, 2013; Obok, 2013; Obok, Aken'Ova, & Iwo, 2012). *Pennisetum purpureum* has also shown potential for use in pull-push pest management strategies to attract stem borer moths (*Coniesta ignefusalis* Hampson.) away from maize (Khan, Midega, Wadhams, Pickett, & Mumuni, 2007). This characteristic can also be used in pearl millet production to protect the crop from stem borer and head minors (*Heliocheilus albipunctella* De Joannis) (Serba, Perumal, Tesso, & Min, 2017). The wild *Pennisetum* species, *P. squamulatum* for apomixis, *P. orientale* L. C. Rich. for apomixis, drought tolerance, perennial growth habit, pest resistance (Dujardin & Hanna, 1987; Hanna & Dujardin, 1982), and *P. schweinfurthii* for improved seed size are potential CWRs for use in pearl millet improvement programs (Table 3). Interspecific hybrids were generated for transferring genes controlling apomixis from *P. squamulatum* into cultivated pearl millet (Dujardin & Hanna, 1983b, 1985, 1989b; Kaushal et al., 2008). Attempts were made to generate interspecific hybrids between pearl millet and other wild species such as *P. orientale* (Dujardin & Hanna, 1983a, 1984a; Hanna & Dujardin, 1982; Kaushal & Sidhu, 2000; Nagesh & Subrahmanyam, 1996; Patil & Singh, 1964; Zadoo & Singh, 1986), *P. mezianum* (Nagesh & Subrahmanyam, 1996), *P. ramosum* (Nagesh & Subrahmanyam, 1996), *P. setaceum* (Forssk.) Chiov. (Hanna, 1979), and *P. schweinfurthii* (Hanna & Dujardin, 1986; Marchais & Tostain, 1997; Nagesh & Subrahmanyam, 1996).

A few wild *Pennisetum* species were also used for developing new cultivars for different purposes. For example, *P. purpureum* and *P. squamulatum* were effectively used in developing ornamental *Pennisetum* cultivars Tift 17 and Tift 23 (Hanna, Braman, & Schwartz, 2010). Tift 17 is a trispecific cross between *P. purpureum*, *P. squamulatum*, and cultivated pearl millet, whereas Tift 23 is an interspecific hybrid between cultivated pearl millet and *P. purpureum*. Since the CWRs of pearl millet occupy drier environments than the cultivated



forms, the introgression of CWR alleles into the cultivated gene pool can lead to an increase in adaptability to harsher conditions (Burgarella et al., 2018). For example, better adaptation of maize genotypes to the lower temperatures and precipitation regime in Mexican highlands is attributed to the introgression of alleles from locally adapted wild maize ecotypes (Concetta et al., 2016). These studies show that the use of wild species can pave a way for the creation and enhancement of useful genetic diversity for further use in pearl millet improvement.

## 5 | THE NEED FOR PREBREEDING: BREEDERS' PERSPECTIVES

The ICRISAT Pearl Millet Genetic Improvement Program, which targets southern Asia and sub-Saharan Africa, has been continuously delivering improved varieties suitable for food, fodder, and feed for the past 45 yr. Pearl millet breeding programs of the Indian public sector, private sector, and ICRISAT have been instrumental in achieving a remarkable increase in pearl millet productivity from 350 kg ha<sup>-1</sup> in 1960 to 1400 kg ha<sup>-1</sup> in 2018 in India, whereas, the African region has an average productivity of 800–900 kg ha<sup>-1</sup> (Bhagavatula et al., 2013). The genetic resources available in the genebank have contributed significantly to crop improvement by providing resistance sources for important abiotic and biotic stresses (Table 3). For example, resistance to downy mildew in pearl millet was identified from 863 B (IP 22303), P 1449-2 (IP 21168), ICMB 90111 (IP 22319), ICMP 451 (IP 22442), and IP 18293 (Upadhyaya, Reddy, & Gowda, 2007). While tremendous progress has been made in improving overall production and productivity, mainly because of a strong focus on yield and yield components, biotic and abiotic stresses still remain a challenge and cause significant yield losses as a result of frequent breakdown of resistance to evolving virulences of pathogens and lack of use of diverse gene pool. Therefore, a continuous flow of novel genes and alleles conferring resistance or tolerance to important biotic and abiotic stresses is required for the incremental and sustainable improvement in the breeding programs. Because of the heavy use of elite × elite germplasm in breeding programs to develop improved high-yielding lines, the gene frequency for defensive traits often gets reduced (Cobb et al., 2019). The susceptibility of high-yielding lines to emerging pests and pathogens is a major concern for the sustainable production of pearl millet. Though some of these diseases can be controlled with chemicals, the integration of native gene variants into improved elite germplasm could be a long-term, cost-effective, and environment-friendly solution. Therefore, concerted efforts are required to explore CWRs to identify and use improved genetic stocks of CWRs in breeding programs for trait improvement (Table 3).

Product profiles and product concept notes have been developed for the improvement of the grain legumes and dry-land cereals including pearl millet in Asia and Africa (Gaur et al., 2019). These profiles and notes have been designed to address the must-have traits (short- to medium-term goals) and the value-added and nice-to-have traits (long-term goals) that allow a breeder to meet market demands, add value, and deliver an incrementally improved variety quickly (Cobb et al., 2019). As pearl millet is grown for different purposes in different agroecologies, lists of the must-have and the value-added traits have been identified for each market segment in Asia and Africa (Gaur et al., 2019). The must-have traits include high yield, early maturity, seed size, improved terminal drought and flowering-stage heat tolerance, resistance to downy mildew and blast, and iron and zinc fortification; and the value-added traits include improved heat (both seedling- and flowering-stage) tolerance, lodging tolerance, forage yield and quality, and rancidity.

Prebreeding can play an important role in improving the must-have traits by providing new and diverse sources of genetic variability to quickly address the current production limitations as well as to address the value-added traits by introgressing the genes and alleles for specific traits from wild species (Table 3), which are not present in the cultivated gene pool. Targeted prebreeding efforts in collaboration with breeders, physiologists, and pathologists will help to address these constraints and will be an important component to enhance the genetic gain in pearl millet. Improving the shelf life of pearl millet flour is an important value-added trait preferred by the market. The shelf life of pearl millet flour is very short because of the rapid development of rancidity caused by the high fat content and lipase activity (Yadav, Anand, Kaur, & Singh, 2012). Breeding programs are not able to generate variability for this complex trait (Mazumdar et al., 2016). A combination of different approaches such as prebreeding using CWRs, genomic tools, and gene editing (CRISPR/Cas) approaches would be very valuable to address the problem of rancidity in pearl millet.

Though the main focus of a crop improvement program is to exploit all available variability within a species and their wild relatives (Maccaferri, Sanguineti, Donini, & Tuberosa, 2003; Palmgren et al., 2015), the frequent use of wild *Pennisetum* species in pearl millet breeding programs is limited because of their adaptation to specific geographical regions as evident from their origin and geographical distribution. Most of the wild *Pennisetum* species are geographically distributed in Africa (Burgarella et al., 2018; Oumar et al., 2008). Western African pearl millet germplasm has high drought tolerance and are late maturing with photoperiod sensitivity, while Indian accessions are mainly early maturing types. This fundamental difference in photoperiod sensitivity has led to the limited use of African germplasm to improve pearl millet lines in India. Therefore, prebreeding can play a crucial role in

using such exotic germplasm to improve the adaptability of pearl millet as reported in maize (Goodman, 2004).

With the advent of genomic tools, breeders can accelerate the breeding cycle and the incorporation of traits based on the availability of trait-specific genes and alleles present in the cultivated backgrounds for use in breeding programs. The use of diagnostic markers will increase the precision and speed of introgression especially for traits that have high genotype  $\times$  environmental interactions. The Pearl Millet  $\sim$ 1,000 Genome Resequencing Project (Varshney et al., 2017) has greatly contributed to pearl millet having a repository of more than 29 million genome-wide single nucleotide polymorphisms (SNPs). This project also resequenced a total of 31 wild *Pennisetum* accessions from *Pennisetum*, which were sampled in the Sahel from Senegal to Sudan. The resequencing provided important information on the nature of genetic diversity of some wild *Pennisetum* species and the relative contraction and expansion of the adaptation and fitness related genes during the domestication history of pearl millet. Because of the available genomic resources, it is possible to know the relative diversity of wild germplasm and the diverse genes and alleles present in pearl millet CWRs. Mariac et al. (2006) had demonstrated usefulness of microsatellite markers for genetic diversity studies. They demonstrated greater allelic diversity and higher number of alleles in 46 wild accessions over 421 cultivated lines from Niger. It will be possible to select beneficial genes and alleles for trait introgression and to functionally characterize the given genes at molecular level before the introgression is initiated. Marker systems based on next-generation sequencing such as whole-genome resequencing SNPs and genotyping-by-sequencing SNPs may help in trait mapping and deployment in appropriate genetic backgrounds. These markers can also help to eliminate the negative linkage drag normally observed in the wide cross derivatives using genome-wide background selection. The available repository of markers can be used to enrich the genetic diversity in pearl millet, which has been lost during the domestication process. Molecular markers can assist genebanks in the molecular characterization and determination of intra- and interaccession variability between different regeneration cycles.

Furthermore, using the next-generation genotyping platforms and the high-end genomic resources, the CWRs of pearl millet can be harnessed to improve the allelic richness of the cultivated germplasm by bringing diversity at the gene, allele, or haplotype levels. These genomic technologies may also be used in precise trait mapping, introgressions, and speed breeding and need to be harnessed in a multidisciplinary way to make pearl millet cultivars resistant to the biotic and abiotic stresses.

Specific areas of interest and need of breeders are as follows, and these core breeding areas can be linked to the use of CWRs to improve pearl millet in the 21st century:

#### (a) CWRs for forage improvement

The use of CWRs to improve pearl millet is evident from the use of *P. purpureum*, also known as Napier grass, in forage pearl millet breeding. Interspecific triploid hybrids between diploid pearl millet ( $2n = 2x = 14$ ) and allotetraploid *P. purpureum* ( $2n = 4x = 28$ ) are widely used in forage breeding. The main objective of crossing these species was to develop high-yielding, high-quality, perennial forage hybrids combining the forage quality and nonshattering nature of pearl millet with the ability to perennial regrowth of Napier grass (Ramamurthy & Shanker, 1998). Several hybrids exhibiting high heterosis in terms of forage yield and quality have been developed using this combination in India and have become popular in well-irrigated areas (Randhawa, Singh, & Sidhu, 1989). However, since these hybrids are triploid, they are sterile, that is, vegetatively propagated and have limited access to remote farmers. This points to prospects for breeding seed-propagated forage hybrids where CWRs could be of potential benefit.

#### (b) Apomixis

Apomixis is a process of asexual reproduction through seed. Apospory, a type of apomixis, occurs in several wild *Pennisetum* species (Ozias-Akins, Roche, & Hanna, 1998). Apomixis is a desirable trait in pearl millet as it can be used to produce true-breeding hybrids (both grain and forage hybrids); fix hybrid vigor regardless of heterozygosity by self-seeding; and enable commercial production of hybrids across generations without CMS, thus making superior hybrid cultivar available at an economic price (Hanna & Bashaw, 1987). If apomixis is introduced into the hybrids with desired heterozygosity and the right gene combinations, it would be possible to perpetuate hybrid vigor over extended periods without having the need to produce hybrid seed every year and distribute to farmers' year after year. Concerted efforts have been made to transfer genes for apomixis from *P. orientale* (Dujardin & Hanna, 1987; Hanna & Dujardin, 1982) into cultivated pearl millet and from *P. squamulatum* ( $2n = 6x = 54$ ) into tetraploid pearl millet through a trispecific hybrid crossing scheme using *P. purpureum* ( $2n = 4x = 28$ ) as a bridge species (Dujardin & Hanna, 1989b, 1989c). Transfer of apomixis from *P. squamulatum* to cultivated pearl millet resulted in an obligate apomictic backcross line with a low but unknown number of chromosomes from the wild species (Ozias-Akins et al., 1993). Molecular markers (restriction fragment length polymorphisms and random amplified polymorphic DNAs) have been identified that unequivocally demonstrate the presence of *P. squamulatum* DNA in the backcross lines (Ozias-Akin et al., 1993). This study suggested that genes for apomixis apparently can be transmitted by a single chromosome. Chromosome-specific markers will

provide a starting point for the mapping of this genetically intractable reproductive trait.

## 6 | STRATEGIES TO ENHANCE USE OF WILD SPECIES FOR CROP IMPROVEMENT

Though wild *Pennisetum* species have many beneficial traits for the genetic improvement of cultivated pearl millet, their use in breeding programs is low because of the cross-incompatibility barriers (both pre- and postzygotic) and linkage drag that hinders the transfer of desirable genes through wide crosses. In the genus *Pennisetum*, incompatibilities between the parental genomes are very common. Gene transfer from tertiary gene pool species is hindered by various pre- and postfertilization barriers including complete male sterility and poor female fertility with obligate apomixis in interspecific hybrids (Dujardin & Hanna, 1989a). Specialized techniques, such as embryo rescue to overcome postzygotic barriers and the use of cross-compatible species as a bridge, are required to gain access to new genes from important GP3 species. In light of these limitations, breeders rely on and prefer elite breeding materials. This may lead to a narrow genetic base and genetic vulnerability of modern crop varieties. Although previous studies have reported on the possibility of producing hybrids between pearl millet and its CWRs, such as *P. mollissimum* and *P. violaceum* and other related species, more efficient use of CWR species to improve crops requires systematic and targeted efforts. These efforts include a deep understanding of the crop, prioritization of traits for CWR use, trait discovery using high-throughput phenotyping and molecular tools, the introgression of traits with minimal linkage drag, and the continuous supply of the new and diverse genetic variability derived from CWRs in the breeding pipeline for further deployment in breeding programs. The success of these efforts depends on careful planning and efficient implementation, frequent monitoring to identify the challenges at each step and measure results and impacts, and strong networking involving cooperation between the public and private sectors.

Prebreeding acts as a bridge between genebanks and breeding programs. It provides an excellent strategy for enhanced use of CWRs and creating new variability through wide hybridization for direct use in breeding programs for the target traits (Figure 4). The genetic diversity conserved in the world's genebanks, particularly for CWRs, is a largely untapped resource for crop improvement (Byrne et al., 2018). Dempewolf et al. (2017), based on consultations with experts from 24 crop communities, described the challenges hindering the increased use of CWRs for crop improvement. These challenges include insufficient phenotypic and genotypic data on CWR accessions, ploidy differences and other

barriers to hybridization between wild species and cultivated germplasm, the presence of sufficient variation in cultivated germplasm of some crops, inferiority of CWRs with respect to desired traits, linkage drag, and insufficient human or financial resources to carry out the necessary research and development. The creative application of technology and appropriate policy changes will help overcome these challenges and unlock the diversity of genebank collections for crop improvement.

The stage-gate process has been proposed for better management of private and public-sector breeding programs involving cultivated  $\times$  cultivated or elite  $\times$  elite crosses (<https://excellenceinbreeding.org/blog/applying-stage-gates-better-manage-public-breeding-programs>). Similar strategy is needed for the efficient management of prebreeding programs using CWRs, and therefore, we hereby propose a common stage-gate process. This stage-gate process, from discovery to delivery, will be applicable to most crops, including pearl millet, and can include the following stages:

**Stage 0. Trait prioritization:** This is a planning phase that involves identifying traits for which there is no or limited variability in the cultivated germplasm in the primary gene pool or there is a need to diversify the genetic base of the traits to minimize genetic vulnerability of modern crop varieties. This is an important phase that requires close cooperation between prebreeders (breeders working with the CWRs and unadapted germplasm), socio-economic scientists, breeders, and genebank managers.

**Stage 1. Trait characterization, validation, and selection of parents:** Once the traits have been prioritized, the next step is to select CWRs and varieties to be used in the crossing program. Passport data can be used for selecting CWRs from genebanks. The preference for selecting CWRs should be in the order of GP1 > GP2 > GP3. Precise characterization and evaluation of CWR species under controlled environmental conditions and in the target population environments (TPEs) using standardized protocols for 2–3 cycles is required to identify diverse, stable, and promising donors. These stable sources, referred as trait-specific genetic stocks (TGSs) of CWRs, will be used as donors in crossing program.

**Stage 2. Germplasm enhancement:** This is concerned with germplasm enhancement of both cultivated and CWR germplasm. Germplasm enhancement of cultivated germplasm involves the generation of successful interspecific  $F_1$  crosses using different TGSs as donors and popular varieties as recipients. Because of the complexity of the traits, it is possible to focus on genetic improvement of CWRs by creating crosses

between diverse TGSs (to combine genes or alleles from different CWRs) using a biparental or more complex multiparental crossing approach.

**Stage 3. Trait discovery:** This includes creating large segregating populations (backcross or filial generations), also known as prebreeding populations, generating knowledge and understanding of the genetics of the trait and identifying diagnostic molecular markers and candidate genes associated with the traits.

**Stage 4. Preliminary testing:** It involves the preliminary evaluation of prebreeding populations for the traits of interest and the identification of promising introgression lines having desirable traits and minimum linkage drag to constitute trait-specific gene pools (TGPs) for multilocation evaluation.

**Stage 5. Multilocation evaluation:** It includes the precise evaluation of selected introgression lines (ILs) in TPEs in collaboration with public- and private-sector partners, and the identification of promising ILs with a high frequency of beneficial genes and alleles introgressed from CWR donors in acceptable agronomic background.

**Stage 6: Trait deployment:** It involves the sharing of promising ILs, TGPs, and prebreeding populations with breeders for further use in breeding programs. As with the breeding programs, this stage-gate process will steer the prebreeding pipeline from design to delivery through a series of stages and decision gates. Such prebreeding programs ensure that new, beneficial, and diverse genetic variability is continuously added to the breeding pipeline in an easily usable form, saving time and money for breeders, reducing risks, and improving efficiency in the development of new, improved varieties with a broad genetic base. Prebreeding using wild species should become an integral part of current breeding programs for all crops and follow the proposed stage-gate process for better management of the prebreeding pipelines.

## 7 | PEARL MILLET PREBREEDING AT ICRISAT

At ICRISAT, efforts are in progress to create new genetic variability by using wild *Pennisetum* species for further use in pearl millet breeding programs. The 305 *P. violaceum* accessions conserved in the genebank were evaluated for blast and rust and resistant accessions were identified. The resistant accessions are being evaluated following pedigree selection to develop TGSs. Using two blast resistant *P. violaceum* accessions (IP 21544 and IP 21720) and four cultivated pearl millet genotypes [one germplasm line (IP 22269), one forage variety (ICMV 05555), and two hybrid parents (ICMB 94555 and

ICMB 97111)], four advanced backcross populations were developed. These populations were derived from four inter-specific crosses: IP 22269 × IP 21544 (designated as Pop 1), ICMV 05555 × IP 21720 (Pop 2), ICMB 94555 × IP 21544 (Pop 3), and ICMB 97111 × IP 21720 (Pop 4). These four populations were evaluated against five diverse pathotype isolates, Pg 45, Pg 138, Pg 186, Pg 204, and Pg 232, of blast pathogen under controlled environmental conditions. Stable pathotype-specific blast resistant ILs have been identified and the TGPs are being constituted. These promising ILs and TGPs will be made available to breeders around the world for use in pearl millet improvement programs. Besides blast resistance, these populations are also being evaluated at target ecologies in India in collaboration with public- and private-sector partners to identify flowering-stage heat and terminal drought tolerant ILs as well as in western and central Africa for resistance to *S. hermonthica*. The promising TGSs, ILs, and TGPs will be conserved in the ICRISAT genebank for future use.

## 8 | OUTLOOK

A wealth of genetic diversity is available in wild *Pennisetum* species in terms of growth habit, forage yield, nutritional quality traits, and resistance or tolerance to biotic and abiotic stresses (Table 3). This hidden and unexplored genetic variation is promising for genetic improvement of both grain and forage pearl millet cultivars and hybrids. Though ~5000 accessions of wild *Pennisetum* species are conserved in 52 genebanks globally, a few accessions have been used in pearl millet breeding programs as is evident from the literature. Targeted and systematic efforts are needed to make prebreeding using CWRs a worthwhile research effort for breeders and to increase the genetic gain of crop varieties. The efficient use of CWRs for crop improvement requires multidisciplinary and cross-institutional cooperation combined with long-term and unrestricted funding. Teams of different experts (pathologist, physiologists, botanists, agronomists, etc.) must support the prebreeding efforts to make grain and forage pearl millet cultivars (both hybrids and open-pollinated varieties) resistant to the biotic and abiotic stresses that are nutritionally dense and suitable for harsh agroecologies and relatively poor management practices in Asia and Africa. In India, >70% of pearl millet production area is cultivated with single-cross hybrids (Yadav & Rai, 2013). Although a number of hybrids are available for better endowed environments, efforts are needed to develop early maturing, dual-purpose hybrids with disease resistance adapted to drought-prone environments in the A<sub>1</sub> zone in India. In western Africa, smallholder farmers prefer open-pollinated varieties because single-cross hybrids do not perform well in the harsh growing conditions of western Africa. As part of South–South collaboration, efforts are



underway to test the adaptability and performance of some promising hybrids from India and western Africa. Prebreeding can play an important role in creating new variability, especially for the adaptation traits, for further use in hybrid breeding programs in western Africa as well as for drought-prone ecologies in India.

Strengthening the public–private partnership is the key factor for a successful prebreeding program. Such partnerships will build on priority setting, exchange of knowledge, integration of technologies, and assistance in mobilizing resources. As the germplasm, including CWRs, is held by public-sector organizations and institutions, the public–private partnership will ensure the private sector’s access to these important resources, and this can be a key factor in delivering the high-quality products derived from the valuable CWRs in the farmers’ fields.

Overall, there is a need to develop a strategic plan for broadening the germplasm base and use of wild *Pennisetum* species for pearl millet improvement. Here are some key action plans, keeping in view the stage-gate process proposed in the previous section, for the efficient use of CWRs for pearl millet improvement:

- (1) The major traits that require prebreeding interventions include identification of new and diverse sources of resistance for blast and striga, broad-spectrum downey mildew resistance, improved tolerance for terminal drought, seedling- and flowering-stage heat tolerance, transferring genes for apomixis, and forage improvement.
- (2) Development of TGSs of CWRs including the following steps:
  - (a) Selection of potential wild *Pennisetum* species for characterization and evaluation

For example, 794 accessions belonging to 26 wild *Pennisetum* species are conserved in the ICRISAT genebank. Based on the passport information and previous reports, the potential wild species can be selected and all the accessions of each species need to be evaluated for target traits. Previous studies indicate that *P. violaceus* for blast, *P. purpureum* for forage improvement, and *P. squamulatum* for apomixis are the potential wild species.

- (b) Develop precise phenotyping techniques for evaluation of photo- and thermo-sensitive CWRs in controlled environmental conditions and TPEs for complex traits showing high genotype  $\times$  environment interactions such as seedling- and flowering-stage heat tolerance.
- (c) Efficient characterization and precise evaluation of wild *Pennisetum* species as potential sources of beneficial variation related to target traits for 2–3 seasons or cycles
- (d) Develop uniform and stable inbred lines of promising wild *Pennisetum* accessions for each target trait, referred

- as TGSs to minimize within accession variability for use in research and conservation in genebanks for future use
- (e) Identify a unique set of TGSs of wild *Pennisetum* accessions based on characterization and passport data and molecular tools to explore genetic diversity
  - (3) Based on cross-compatibility relationship between cultivated pearl millet and wild species, initiate a systematic prebreeding program using diverse TGSs as donors and popular, widely adapted cultivars as recipients. Specialized techniques, such as embryo rescue, bridge species, are required to introgress genes and alleles from cross-incompatible GP3 species such as *P. schweinfurthii*, *P. mezianum*, *P. ramosum*, and others. In addition, diverse TGSs belonging to the same or different species may also be crossed to generate new and promising recombinations for further use as new donors in germplasm enhancement of cultivated pearl millet.
  - (4) Development of large-sized prebreeding populations followed by genotyping and phenotyping of prebreeding population to study the genetics of the traits and marker–trait associations.
  - (5) Preliminary evaluation of prebreeding populations for traits of interest for one to two seasons, preferably under controlled environmental conditions, to identify promising ILs with traits of interest introgressed from CWR species in acceptable agronomic background. The main objective of pearl millet improvement is to develop extra-early and early elite material with acceptable agronomic performance that fits into short cropping seasons. Hence, selection can be made for earliness during this evaluation phase.
  - (6) Evaluation of selected ILs and prebreeding populations in different TPEs, preferably in a public–private partnership, and identification of stable and promising ILs with better adaptability to constitute TGP.
  - (7) Sharing stable and promising ILs and TGPs with public- and private-sector breeding programs for use as sources of new and diverse variations in mainstream breeding program as well as conservation in genebanks for future use.

Further, using the next-generation genotyping platforms and the latest genomic resources, the CWRs of pearl millet can be harnessed to improve the allelic richness of the cultivated germplasm by increasing diversity at the gene, allele, or haplotype level. Genomic tools can be used to identify and rapidly transfer and track the introgressed fragments into cultivated background. These genomic technologies can be used for precise trait mapping and introgressions. The development of the pangenome of different wild species within the genus, referred to as a ‘super-pangenome,’ will be useful for cataloguing the entire genome repertoire of a genus (Khan et al., 2020), mainly contributed by structural variations, and will improve the precision and efficiency of trait introgression



by prebreeding using CWRs for crop improvement. Diverse TGSs will serve as the most appropriate material for the development of the super-pangenome and will be useful to capture the maximum diversity for a species vis-à-vis minimizing the within-accession variability present in the germplasm conserved in genebanks.

Because of its superior tolerance to drought, high temperature, and salinity compared with many other cereals and having short duration and high nutritional value, pearl millet has the potential to occupy new niches around the world particularly in the semi-arid zones of central Asia and the Middle East, North and South America, and Australia (National Research Council, 1996).

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## CONFLICT OF INTEREST

The authors have no conflict of interests to declare.

## AUTHOR CONTRIBUTIONS

S. Sharma: Conceptualization, writing—status of the crop wild relatives and strategies to enhance the use of CWRs for pearl millet improvement. R. Sharma: writing—use of CWRs for biotic stresses. M. Govindaraj, R.S. Mahala & C. Tara Satyavathi: writing—breeders’ perspectives for prebreeding. R.K. Srivastava: writing—genomic resources. M.K. Gumma: geo-spatial analysis. B. Kilian: writing, specifically, review & editing. All authors reviewed and approved the final manuscript.

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## SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of the article.

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