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## *Genetic Resources Communication*

# Genetic diversity and population structure of *Heteropogon contortus* L. germplasm collected from diverse agro-climatic regions in India and development of a core germplasm set

## *Diversidad genética y estructura poblacional de germoplasma de* Heteropogon contortus *L. provenientes de regiones agro-climáticas diversas en India y desarrollo de una colección núcleo*

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### Abstract

*Heteropogon contortus*, an important constituent of major grasslands of India, Australia and many countries in Africa, Asia and the Americas, is important for pasture and grassland productivity. Hence genetic improvement of the grass needs attention. A genetic variability study, including development of a core subset, was carried out by evaluating 235 accessions collected from different agro-ecological zones of India. The study, based on 16 metric and 14 non-metric traits along with 8 nutritional parameters, indicated that considerable genetic variability existed among the germplasm and selection could result in identification of suitable types for target environments. Clustering and subclustering was performed to select 35 accessions to form a core subset. The statistical analysis indicated that the core subset captured almost all the variability present in the entire germplasm. The study will help researchers to focus future studies on this core subset in developing genetic improvement programs.

**Keywords:** Black spear grass, forage grass, grassland, plant genetic resources, rangeland.

### Resumen

*Heteropogon contortus* es una pastura altamente utilizada en la India, Australia y muchos países de África, Asia y América; muy importante para la productividad forrajera. Por tanto, es necesario prestar atención al mejoramiento genético de la hierba. Se llevó a cabo un estudio de variabilidad genética, incluido el desarrollo de un subconjunto central, mediante la evaluación de 235 accesiones recolectadas de diferentes zonas agroecológicas de la India. El estudio, basado en 16 rasgos métricos y 14 no métricos junto con 8 parámetros nutricionales, indicó que existía una variabilidad genética considerable entre el germoplasma y que la selección podría resultar en la identificación de tipos adecuados para los ambientes objetivo. Se realizaron agrupaciones y subgrupos para seleccionar 35 accesiones para formar un subconjunto central. El análisis estadístico indicó que el subconjunto central capturó casi toda la variabilidad presente en todo el germoplasma. El estudio ayudará a los investigadores a centrar los estudios futuros en este subconjunto central en el desarrollo de programas de mejora genética.

**Palabras clave:** Barba negra, gramínea forrajera, pasturas, recursos genéticos, variabilidad.

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## Introduction

*Heteropogon contortus* (L.) P. Beauv. ex Roem. & Schult. (Poaceae: Andropogoneae) is an important perennial pasture grass. It is commonly known as black spear grass or bunch spear grass (Australia), tangle head (United States), pilli grass (Hawaii), assegai grass (Zimbabwe) and Lampa ghas (India). The grass is native to Africa, southern Asia, northern Australia and parts of Oceania and is naturalized in tropical and subtropical regions of the Americas, East Asia and Oceania. Commonly found in tropical Africa (Soromessa 2011), it is also very common in other tropical, subtropical and warm temperate regions of the world, particularly in the Indian subcontinent, Burma, North Africa, Australia and the Pacific as a perennial range grass. The grass is frequently present in major grasslands of India such as *Sehima-Dichanthium, Dichanthium-Cenchrus-Lasiurus, Themeda-Arundinella* and Peninsular India grasslands (Dabadghao and Shankarnarayan 1973; Malaviya et al. 2018; Malaviya and Roy 2021) and establishes well around tropical and subtropical grasslands of the world (Carino 1999).

*H. contortus* is an important shrub-layer grass of moist deciduous forests (Rhind 2010) dominated by *Shorea robusta* in the upper Gangetic plains (Singh 2012). Historically, Hawaiians used fire to manage grasslands dominated by *H. contortus* (Hoffmann 2008; Daehler and Goergen 2005), although Goergen and Daehler (2001) reported the grass to be only lowly tolerant of clipping and burning. It has also been found to be an effective live mulch in tropical agricultural systems (Moata 2009). *H. contortus*, the dominant grass in northern Australia savanna, is a valued species for cattle, but noxious for sheep because of its awns and a weed in sown grass-legume pastures (FAO 2007).

*H. contortus* prefers areas with rainfall less than 800 mm, is adapted to coarse-textured soils with pH 6–8 and has moderate to low tolerance of drought, high temperatures, soil salinity and low soil fertility (Moata 2009). Fang and Xiong (2015) and Wang et al. (2016) indicated that, although the grass is drought-tolerant, severe water stress inhibits its growth. Due to its tolerance of soil nutrient deficiencies and limited soil moisture, it is a valuable species for vegetation restoration (Goergen and Daehler 2001; 2002). Owing to its high nutritional and medicinal value, Daehler and Goergen (2005) considered ethnobotanical and ecological research was important for restoration of *H. contortus*-dominated grasslands. *H. contortus* is tolerant of limited shading and often dominates the under-storey of woodlands in tropical and subtropical Australia.

The grass is a nutritious fodder which can be easily conserved as hay (Bor 1960). It is highly palatable during the vegetative phase. However, its robust awns shed easily and are considered a negative factor since at maturity they are pointed sharp and intermingle, causing injury in the mouth and stomach of grazing animals. It spreads naturally and is considered an aggressive invasive species because its seeds survive even after burning of rangelands/grasslands by burying themselves (Roy et al. 2019a).

In India, livestock production is primarily based on rangeland and grassland grazing. About 40% of the country is available for grazing of livestock as pasture lands, forest lands, cultivated wastelands, fallow lands, non-agricultural lands, miscellaneous tree crops and groves etc. In such grazing lands in tropical, subtropical, arid, semi-arid and lower hills of India and isoclimatic conditions in the world, *H. contortus* is widely adapted. However, to increase pasture productivity it is essential to introduce high-yielding accessions in such grazing lands, especially in dry areas. There is a deficit in demand and supply of green and dry forages for livestock in India (Roy et al. 2019b) and perennial grasses like *Heteropogon* are important in bridging the gap.

At an international level, genetic diversity of the grass is conserved in germplasm collections at the Southern Regional Plant Introduction Station, Griffin, Georgia of the USDA/ARS National Genetic Resources Program, and in Indonesia, Ethiopia and Zimbabwe; however, there is negligible representation of germplasm from the Indian subcontinent. ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India (ICAR-IGFRI) has collected a wide diversity of this grass through a series of explorations from various agro-climatic conditions in the country. The germplasm is maintained at ICAR-IGFRI and ICAR-National Bureau of Plant Genetic Resources, New Delhi, India. Knowledge about the existing genetic variability is a prerequisite for any genetic improvement program, and studies of available natural variation are helpful in developing a genetic improvement strategy. Hence, the present study was undertaken to critically evaluate available germplasm, collected from diverse parts of India, to enable breeders to utilize the genetic divergence for isolating promising types. Additionally, development of a core germplasm subset was envisaged, to allow researchers to focus on a limited range of germplasm for further studies and also to accelerate breeding programs.

## Material and Methods

A study of variability was conducted on 235 accessions of *H. contortus* collected from different agro-ecological zones of India and conserved at ICAR-IGFRI, Jhansi, India. A list of accessions along with their place of collection is given in Table 1. The soil and climatic conditions of the place of collection following agro-ecological zones defined by Ahmad et al. (2017) and ICAR (2018), are presented in Table 2.

Seeds of the accessions were taken from the gene bank of IGFRI and planted in a nursery. Six-week-old seedlings were transplanted in 3 m-long paired rows, spaced at 75 cm, accommodating 6 tussocks in each row. Rows of plants were 1 m apart. Data on morphological traits were recorded at 50% flowering stage on 3 randomly-selected plants, excluding border plants at the ends of the rows. Seed-related observations were recorded at maturity.

Data on 16 quantitative metric traits were recorded, i.e. length of main tiller (cm); number of tillers/tussock; fresh weight (g) of single tussock (average of 3) harvested at 50% flowering; main tiller diameter (cm); internodal length (cm); number of nodes on main tiller; leaf blade length (cm); leaf blade width (cm); leaf sheath length (cm); leaf sheath width (cm); flag leaf blade length (cm); flag leaf blade width (cm); flag leaf sheath length (cm); flag leaf sheath width (cm); ligule length (mm); and inflorescence length (cm). Length of the main tiller was measured from ground-level to the tip of the last emerged leaf. The fourth leaf from the top was considered for measuring leaf width and length. Internodal length was measured between 3rd and 4th nodes.

Data were also recorded for 14 non-numeric traits, i.e. growth (overall vegetative growth by visual observation as very poor, poor, medium, good, very good), habit (prostrate, decumbent, erect), leaf hairiness (glabrous, light hairy, hairy), leaf blade color (light green, green, bluish green, greenish blue, greenish violet), leaf sheath color (light green, green, greenish violet), tiller internode color (light green, greenish violet, light violet, violet), node anthocyanin coloration (light violet, violet, dark violet), node color (green, greenish violet, violet, dark violet), node hairiness (light hairy, non-hairy), anthocyanin coloration on leaf sheath (absent, weak, strong), anthocyanin coloration on leaf blade (absent, weak, medium, strong), spike color (light green, green, greenish violet), awn stature (hard, soft) and awn pubescence distribution (pubescence up to 50% awn length, pubescence on full awn length i.e. 100%).

Nutritional quality parameters were analyzed for 167 accessions in terms of: ash (%); organic matter (%); neutral detergent fiber (NDF) (%); acid detergent fiber (ADF) (%); hemicellulose (%); cellulose (%); lignin (%); and crude protein concentrations (%). AOAC (1980) methods were followed for estimation of dry matter, crude protein and ash, whereas the Goering and Van Soest (1970) method was followed for NDF and ADF. In vitro dry matter digestibility (IVDMD) % was estimated following Tilley and Terry (1963) for 81 accessions. For these analyses, samples were collected on different dates, so as to match a uniform 50% flowering stage (i.e. 50% of the inflorescence in the row having spikelets at the anthesis stage). The whole plant was harvested from 10 cm above ground and the samples were oven dried at 60 °C and ground.

### *Statistical analysis*

The metric traits data (excluding ligule length) were averaged over replications and analyzed statistically using Non–Hierarchical Euclidian Cluster Analysis of grouping of accessions (Sparks 1973). The computer software 'Statistical Tool for Agricultural Research (STAR)' (IRRI 2020) was used for computation. The accessions per cluster for the core subset were decided upon following Brown (1989a). The number of accessions for a core subset was kept at 15% of the total germplasm following Brown (1989a). Accessions from each cluster were selected as per the formula below, following Roy et al. (2020) and the logarithmic strategy suggested by Brown (1989b). Accessions for the core subset from each cluster were randomly selected.

$$s = (\log p^{i} / m\Sigma^{t=1} \log p) \times n$$

where:

$s$ = number of accessions selected in a character;

$p$ = size of cluster;

$p^{i}$ = proportion of ith cluster;

$n$ = number of accessions to be selected for core (15% of base collection); and

$m$ = total number of clusters.

For larger clusters, a second level of clustering was done for further assortment of accessions, to constitute the core subset.

Correlation among various quantitative traits was studied using Microsoft Excel program (MS-Excel). Key characters contributing to diversity were identified using Principal Component Analysis. Scree plot analysis was performed to determine the number of principal components to be retained as per Cattell (1966). The mean value for different traits, as obtained in the core value, was compared with that of total germplasm by student's t-test using MS-Excel.

**Table 1.** List of accessions of *Heteropogon contortus* and their place of collection (state in India).

| SN | Accession | State | SN | Accession | State | SN | Accession | State | SN | Accession | State |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | Bundel var-1 | MP | 60 | IG02-635 | Cg | 119 | IG95-13 | UP | 178 | IG97-167 | MP |
| 2 | Hc-13 | UP | 61 | IG02-636 | Cg | 120 | IG95-15 | UP | 179 | IG97-181 | MP |
| 3 | Hc-15 | UP | 62 | IG02-637 | Cg | 121 | IG95-17 | UP | 180 | IG97-182 | MP |
| 4 | Hc-17 | UP | 63 | IG02-639 | Cg | 122 | IG95-21 | UP | 181 | IG97-183 | MP |
| 5 | Hc-18 | UP | 64 | IG02-640 | Cg | 123 | IG95-23 | UP | 182 | IG97-183 | MP |
| 6 | Hc-23 | UP | 65 | IG02-641 | Mh | 124 | IG95-242 | MP | 183 | IG97-183 | MP |
| 7 | Hc-5/18 | UP | 66 | IG02-641 | Mh | 125 | IG95-242 | MP | 184 | IG97-209 | MP |
| 8 | IG01-513 | MP | 67 | IG02-642 | Mh | 126 | IG95-25 | UP | 185 | IG97-209 | MP |
| 9 | IG01-514 | MP | 68 | IG02-643 | Mh | 127 | IG95-258 | MP | 186 | IG97-224 | MP |
| 10 | IG01-516 | MP | 69 | IG02-644 | Mh | 128 | IG95-26 | UP | 187 | IG97-253 | MP |
| 11 | IG01-517 | MP | 70 | IG02-645 | Mh | 129 | IG95-270 | MP | 188 | IG99-210 | Rj |
| 12 | IG01-519 | MP | 71 | IG02-646 | Mh | 130 | IG95-271 | MP | 189 | IG99-210 | Rj |
| 13 | IG01-520 | MP | 72 | IG02-647 | Mh | 131 | IG95-274 | MP | 190 | IG99-219 | Rj |
| 14 | IG01-520 | MP | 73 | IG02-649 | Mh | 132 | IG95-277 | MP | 191 | IG99-311 | Rj |
| 15 | IG01-522 | MP | 74 | IG02-650 | Mh | 133 | IG95-279 | MP | 192 | IG99-312 | Rj |
| 16 | IG01-522 | MP | 75 | IG02-651 | Mh | 134 | IG95-280 | MP | 193 | IG99-313 | Rj |
| 17 | IG02-129 | UP | 76 | IG02-652 | Mh | 135 | IG95-283 | MP | 194 | IG99-314 | Rj |
| 18 | IG02-185 | UP | 77 | IG02-653 | Mh | 136 | IG95-284 | MP | 195 | IG99-314 | Rj |
| 19 | IG02-190 | UP | 78 | IG02-655 | Mh | 137 | IG95-284 | MP | 196 | IG99-315 | Rj |
| 20 | IG02-191 | UP | 79 | IG02-655 | Mh | 138 | IG95-286 | MP | 197 | IG99-316 | Rj |
| 21 | IG02-195 | UP | 80 | IG02-657 | Mh | 139 | IG95-287 | MP | 198 | IG99-317 | Rj |
| 22 | IG02-204 | UP | 81 | IG02-657 | Mh | 140 | IG95-289 | MP | 199 | IG99-318 | Rj |
| 23 | IG02-205 | UP | 82 | IG02-658 | MP | 141 | IG95-290 | MP | 200 | IG99-319 | Rj |
| 24 | IG02-209 | UP | 83 | IG02-658A | MP | 142 | IG95-292 | MP | 201 | IG99-319 | Rj |
| 25 | IG02-291 | HP | 84 | IG02-659 | MP | 143 | IG95-293 | MP | 202 | IG99-320A | Rj |
| 26 | IG02-293 | HP | 85 | IG02-660 | MP | 144 | IG95-328 | MP | 203 | IG99-321 | Rj |
| 27 | IG02-342 | MP | 86 | IG02-661 | MP | 145 | IG95-340 | MP | 204 | IG99-322 | Rj |
| 28 | IG02-343 | MP | 87 | IG02-663 | MP | 146 | IG95-341 | UP | 205 | IG99-323 | Rj |
| 29 | IG02-344 | MP | 88 | IG02-665 | MP | 147 | IG95-341 | UP | 206 | IG99-325 | Rj |
| 30 | IG02-345 | MP | 89 | IG02-666 | MP | 148 | IG95-343 | UP | 207 | IG99-326 | Rj |
| 31 | IG02-346 | MP | 90 | IG02-668 | MP | 149 | IG95-344 | UP | 208 | IG99-327 | Rj |
| 32 | IG02-347 | MP | 91 | IG02-670 | MP | 150 | IG95-344 | UP | 209 | IG99-329 | Rj |
| 33 | IG02-348 | MP | 92 | IG02-671 | MP | 151 | IG95-345 | UP | 210 | IG99-330 | Rj |
| 34 | IG02-349 | MP | 93 | IG02-679 | MP | 152 | IG95-346 | UP | 211 | IG99-333 | Rj |
| 35 | IG02-350 | MP | 94 | IG03-361 | TN | 153 | IG95-346 | UP | 212 | IG99-335 | Rj |
| 36 | IG02-351 | MP | 95 | IG03-371 | Kl | 154 | IG95-347 | UP | 213 | IG99-336 | Rj |
| 37 | IG02-352 | MP | 96 | IG03-371 | Kl | 155 | IG95-348 | UP | 214 | IG99-337 | Rj |
| 38 | IG02-353 | MP | 97 | IG03-376 | Kl | 156 | IG95-349 | UP | 215 | IG99-338 | Rj |
| 39 | IG02-354 | MP | 98 | IG03-377 | Kl | 157 | IG95-350 | UP | 216 | IG99-338 | Rj |
| 40 | IG02-355 | MP | 99 | IG2000-101 | UP | 158 | IG95-352 | UP | 217 | IG99-345 | MP |
| 41 | IG02-356 | MP | 100 | IG2000-73 | UP | 159 | IG95-352 | UP | 218 | IG99-346 | MP |
| 42 | IG02-357 | UP | 101 | IG2000-73A | UP | 160 | IG95-363 | UP | 219 | IG99-349 | MP |
| 43 | IG02-358 | UP | 102 | IG2000-74 | MP | 161 | IG95-366 | UP | 220 | IG99-50 | Cg |
| 44 | IG02-359 | UP | 103 | IG2000-93 | MP | 162 | IG95-367 | UP | 221 | IG99-51 | Cg |
| 45 | IG02-362 | UP | 104 | IG2000-98 | UP | 163 | IG95-368 | UP | 222 | IGO2-184 | UP |
| 46 | IG02-363 | J&K | 105 | IG95-101 | MP | 164 | IG95-369 | UP | 223 | IGO2-186 | UP |
| 47 | IG02-364 | J&K | 106 | IG95-103 | MP | 165 | IG95-369 | UP | 224 | IGO2-187 | UP |
| 48 | IG02-371 | MP | 107 | IG95-104 | MP | 166 | IG95-371 | UP | 225 | IGO2-188 | UP |
| 49 | IG02-375 | MP | 108 | IG95-104-1 | MP | 167 | IG95-374 | UP | 226 | IGO2-189 | UP |
| 50 | IG02-487 | MP | 109 | IG95-104-2 | MP | 168 | IG95-374 | UP | 227 | IGO2-191 | UP |
| 51 | IG02-624 | MP | 110 | IG95-104A | MP | 169 | IG95-7 | UP | 228 | IGO2-192 | UP |
| 52 | IG02-625 | MP | 111 | IG95-104B | MP | 170 | IG95-99 | MP | 229 | IGO2-193 | UP |
| 53 | IG02-626 | Mh | 112 | IG95-105 | MP | 171 | IG96-164 | Kt | 230 | IGO2-200 | UP |
| 54 | IG02-627 | Mh | 113 | IG95-105A | MP | 172 | IG96-167 | Kt | 231 | IGO2-201 | UP |
| 55 | IG02-629 | Mh | 114 | IG95-106 | MP | 173 | IG96-21 | TN | 232 | IGO2-281 | HP |
| 56 | IG02-630 | Mh | 115 | IG95-108 | MP | 174 | IG96-97 | TN | 233 | IGO2-281 A | HP |
| 57 | IG02-631 | Mh | 116 | IG95-109 | MP | 175 | IG97-163 | MP | 234 | IGO2-288 | HP |
| 58 | IG02-632 | Mh | 117 | IG95-110 | MP | 176 | IG97-165 | MP | 235 | IGO2-294 | HP |
| 59 | IG02-633 | Mh | 118 | IG95-111 | MP | 177 | IG97-166 | MP | | | |

Cg = Chhattisgarh; HP = Himachal Pradesh; J&K = Jammu & Kashmir; Kl = Kerala; Kt = Karnataka; MP = Madhya Pradesh; Mh = Maharashtra; Rj = Rajasthan; TN = Tamil Nadu; UP = Uttar Pradesh.

**Table 2.** Soil and climate conditions in the Indian states where accessions of *Heteropogon contortus* were collected [Source: Ahmad et al. (2017); ICAR (2018)].

| SN | State | Climate | Precipitation (mm/yr) | PET (mm) | CGP | Soil |
|---|---|---|---|---|---|---|
| 1 | Rajasthan | Hot arid/semi-arid with desert | <300–800 | 1,500–2,000 | <90 | Saline, alluvium-derived |
| 2 | Maharashtra | Hot semi-arid to hot subhumid | 600–1,000 | 1,600-1,800 | 90–180 | Shallow and medium (with inclusion of deep) black; black & red |
| 3 | Madhya Pradesh | Hot semi-arid to hot subhumid | 500–1,000 | 1,400–2,000 | 90–180 | Alluvium-derived soils or medium and deep black soils |
| 4 | Karnataka | Hot subhumid to semi-arid | 600–1,000 | 1,300–1,600 | 90–150 | Coastal alluvium-derived soils;shallow and medium (with inclusion of deep) black, red loamy |
| 5 | Himachal Pradesh | Warm subhumid to humid, also with some perhumid zone | 1,600–2,000 | 800–1,300 | 180–210+ | Brown forest and podzolic soils |
| 6 | Jammu & Kashmir | Warm subhumid to humid also with some perhumid zones | 1,600–2,000 | 800–1,300 | 180–210+ | Brown forest and podzolic soils |
| 7 | Chhattisgarh | Hot subhumid | 1,200–1,600 | 1,400–1,500 | 150–180 | Red and yellow soils |
| 8 | Tamil Nadu | Hot semi-arid | 600-1,000 | 1.300–1,600 | 90–150 | Red loamy soils |
| 9 | Uttar Pradesh | Hot subhumid | 1,000–1,500 | 1,300–1,500 | 90–180 | Red and black soils, alluvium-derived |
| 10 | Kerala | Hot humid, perhumid | 2,000–3,200 | 1,400–1,600 | 90–210+ | Red, lateritic and alluvium-derived soils |

PET = Potential Evapotranspiration; CGP = Crop growing period (no. of days)

## Results

Observations were recorded on 235 accessions of *H. contortus* for 16 numeric and 14 non-numeric morphological traits and on 167 accessions for 9 nutritional quality parameters in order to characterize the germplasm being maintained at the IGFRI gene bank, and to further develop a core subset of germplasm. Clustering of accessions using 15 metric traits (excluding ligule length) resulted in formation of 6 distinct clusters (Figure 1, Table 3). Clusters 3 and 4 were big clusters consisting of 101 and 94 accessions, respectively, whereas Cluster 1 was of moderate size comprising 30 accessions. Clusters 2, 5 and 6 were small clusters, comprising 3, 5 and 2 accessions, respectively. In Cluster 6, the 2 accessions were vigorous with high values for fresh weight, tiller height, tiller diameter, leaf length and leaf width. These two accessions originated from north and central India, representing tropical climate conditions. The accessions of Cluster 2 also showed high values for agronomic traits. Other clusters showed moderate values for various agronomic traits. Collections from the State of Uttar Pradesh were from the semi-arid districts near Jhansi and the majority of these 63 accessions grouped in Clusters 3 and 4, with a few in Clusters 1, 5 and 6. Similarly, 29 accessions from the arid climate of Rajasthan (western Indian state) grouped mostly in Clusters 3 and 4. Barring 2 accessions, the remaining accessions were from temperate environments, i.e. Himachal Pradesh and Jammu and Kashmir, grouped in Cluster 3; however, this cluster included also accessions from the hot arid climates of central, western and northern India.

Principal Component Analysis revealed that the first 6 principal components accounted for more than 80% of the cumulative variability (Table 4). Scree plot analysis revealed that the first 2 principal coordinates or up to 6 principal components can be retained for explaining most of the diversity (Figure 2).

Height of the plants ranged from 36 to 110 cm (mean 74 cm) (Table 4). Accessions with poor tillering and shorter plant heights were generally annual types. Robust accessions possessed as many as 265 tillers, whereas the minimum tiller number per tussock was 9 only. These 2 traits contribute significantly to total biomass, which was well reflected in fresh biomass, which ranged from 20 to 435 g. The highest fresh biomass per tussock was noted for Cluster 6 (263 g) with 2 accessions only. The second highest average fresh biomass per tussock occurred in accessions of Cluster 3 (143 g), followed by that of Cluster 4 (125 g). Morphological traits, which showed highly significant ($P<0.01$) positive correlation with fresh biomass, were plant height (0.299), number of tillers (0.758) and leaf length (0.289). Tiller diameter with 0.130 and leaf width with 0.140 correlation coefficients were also positively significant ($P<0.05$). However, internodal length was not correlated with fresh

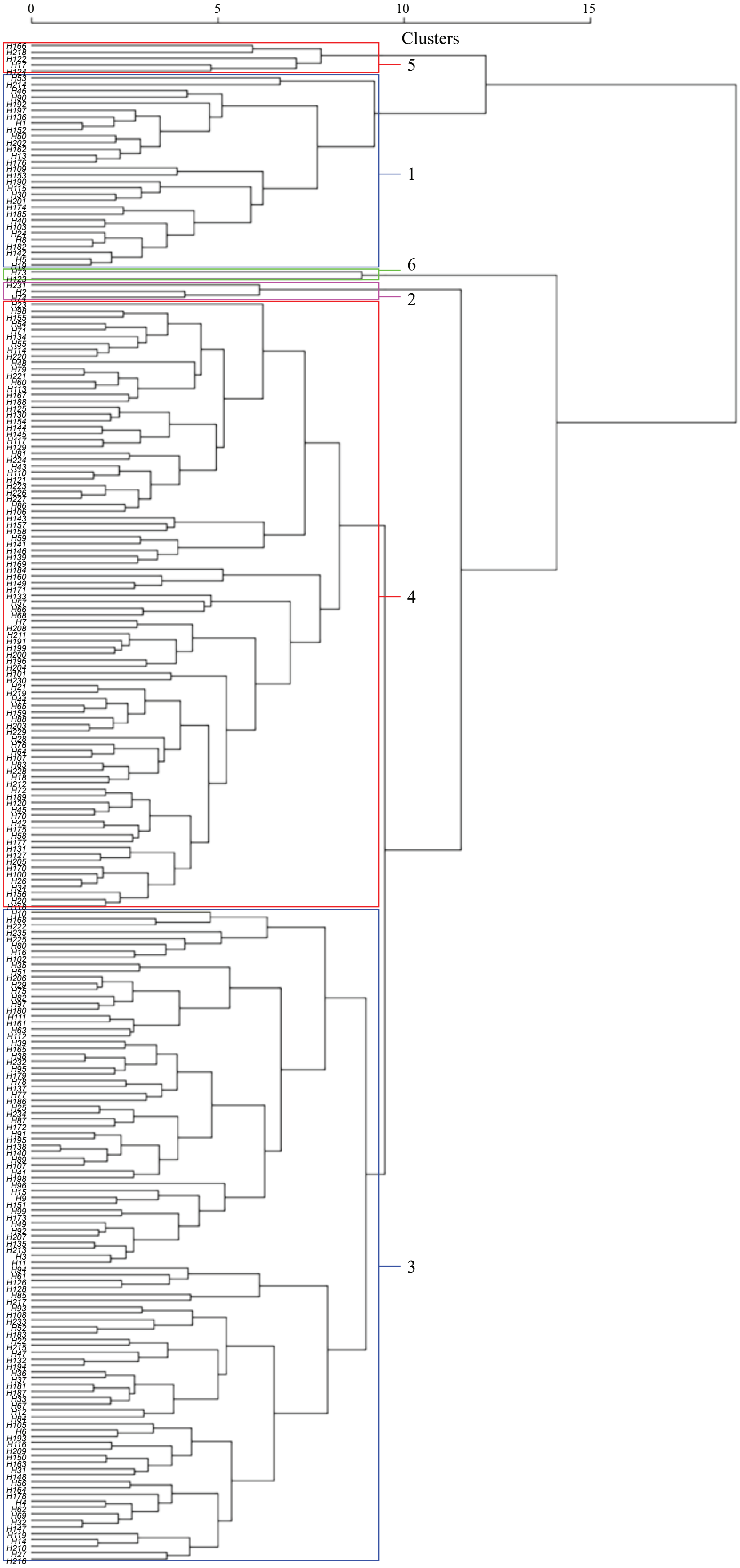

**Figure 1**. Clustering of *Heteropogon contortus* accessions (dendrogram using agglomerative clustering method).

**Table 3.** Accessions of *Heteropogon contortus* belonging to different clusters.

| Cluster | Accessions (as per SN in Table 1) |
|---|---|
| 1 | 1, 5, 8, 13, 19, 24, 30, 40, 46, 50, 53, 90, 103, 109, 115, 136, 142, 152, 153, 162, 174, 176, 182, 185, 190, 192, 197, 201, 202, 214 |
| 2 | 2, 74, 231 |
| 3 | 3, 4, 6, 9, 10, 11, 12, 14, 15, 16, 22, 25, 27, 29, 31, 32, 33, 35, 36, 37, 38, 39, 41, 47, 49, 51, 52, 56, 61, 62, 63, 67, 69, 75, 77, 78, 80, 82, 84, 85, 87, 89, 91, 92, 93, 94, 95, 96, 97, 99, 102, 105, 107, 108, 111, 112, 116, 119, 126, 128, 132, 135, 137, 138, 140, 147, 148, 150, 151, 161, 163, 164, 165, 168, 172, 173, 178, 179, 180, 181, 183, 186, 187, 193, 194, 195, 198, 206, 207, 209, 210, 213, 215, 216, 217, 222, 225, 232, 233, 234, 235 |
| 4 | 7, 18, 20, 21, 23, 26, 28, 34, 42, 43, 44, 45, 48, 54, 55, 57, 58, 59, 60, 64, 65, 66, 68, 70, 71, 72, 76, 79, 81, 83, 86, 88, 98, 100, 101, 104, 106, 110, 113, 114, 117, 118, 120, 121, 125, 127, 129, 130, 131, 133, 134, 139, 141, 143, 144, 145, 146, 149, 154, 155, 156, 157, 158, 159, 160, 167, 169, 170, 171, 175, 177, 184, 188, 189, 191, 196, 199, 200, 203, 204, 205, 208, 211, 212, 219, 220, 221, 223, 224, 226, 227, 228, 229, 230 |
| 5 | 17, 122, 124, 166, 218 |
| 6 | 73, 123 |

**Table 4.** Descriptive statistics of 15 quantitative metric traits among *Heteropogon contortus* accessions, cluster mean performances and principal components.

| | C1 | C2 | C3 | C4 | C5 | C6 | C7 | C8 | C9 | C10 | C11 | C12 | C13 | C14 | C15 |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Descriptive statistics of traits among total accessions | | | | | | | | | | | | | | | |
| Min | 36.2 | 9.00 | 20.0 | 0.10 | 3.73 | 3.00 | 3.50 | 0.30 | 3.27 | 0.30 | 1.07 | 0.10 | 3.90 | 0.10 | 2.67 |
| Max | 110.7 | 265.0 | 435.0 | 0.34 | 14.1 | 13.0 | 29.0 | 1.03 | 9.00 | 1.03 | 10.7 | 0.63 | 12.0 | 0.50 | 12.0 |
| average | 74.3 | 74.3 | 125.6 | 0.19 | 8.32 | 5.39 | 14.1 | 0.60 | 5.96 | 0.60 | 6.50 | 0.35 | 7.16 | 0.35 | 5.64 |
| SD | 12.61 | 45.38 | 72.93 | 0.04 | 1.91 | 1.43 | 3.64 | 0.11 | 0.92 | 0.11 | 1.51 | 0.06 | 1.14 | 0.06 | 0.98 |
| Kurtosis | 0.23 | 2.32 | 2.51 | 2.13 | -0.09 | 5.34 | 1.69 | 1.25 | 1.10 | 1.25 | 0.82 | 2.17 | 1.49 | 1.02 | 12.78 |
| Skewness | 0.22 | 1.28 | 1.26 | 0.44 | -0.13 | 1.83 | 0.72 | 0.25 | 0.56 | 0.26 | -0.26 | -0.16 | 0.36 | -0.53 | 2.11 |
| Cluster means of traits | | | | | | | | | | | | | | | |
| I | 61.0 | 51.8 | 69.8 | 0.16 | 8.17 | 4.67 | 9.63 | 0.48 | 5.16 | 0.48 | 5.15 | 0.30 | 6.51 | 0.30 | 5.06 |
| II | 95.3 | 66.1 | 109.7 | 0.22 | 9.17 | 5.89 | 18.9 | 0.70 | 7.27 | 0.70 | 6.88 | 0.36 | 8.36 | 0.37 | 11.10 |
| III | 72.6 | 90.1 | 143.1 | 0.18 | 8.73 | 4.93 | 13.7 | 0.57 | 5.87 | 0.57 | 6.26 | 0.34 | 7.02 | 0.34 | 5.48 |
| IV | 79.1 | 65.8 | 124.9 | 0.21 | 7.89 | 6.00 | 15.5 | 0.67 | 6.17 | 0.67 | 7.38 | 0.38 | 7.35 | 0.38 | 5.85 |
| V | 72.2 | 56.5 | 77.1 | 0.17 | 9.22 | 5.00 | 14.6 | 0.51 | 6.92 | 0.51 | 2.39 | 0.18 | 9.67 | 0.21 | 5.49 |
| VI | 107.0 | 72.5 | 262.5 | 0.34 | 7.00 | 11.5 | 28.7 | 0.90 | 8.35 | 0.90 | 6.65 | 0.35 | 6.85 | 0.35 | 4.8 |
| Principal Components | | | | | | | | | | | | | | | |
| SD | 2.23 | 1.46 | 1.35 | 1.17 | 0.99 | 0.93 | 0.83 | 0.73 | 0.71 | 0.63 | 0.49 | 0.44 | 0.42 | 0.31 | 0.07 |
| VP | 0.33 | 0.14 | 0.12 | 0.09 | 0.07 | 0.06 | 0.05 | 0.04 | 0.03 | 0.03 | 0.02 | 0.01 | 0.01 | 0.01 | 0.00 |
| CP | 0.33 | 0.48 | 0.60 | 0.69 | 0.75 | 0.81 | 0.86 | 0.89 | 0.93 | 0.95 | 0.97 | 0.98 | 0.99 | 1.00 | 1.00 |
| EV | 4.99 | 2.14 | 1.83 | 1.38 | 0.97 | 0.86 | 0.69 | 0.53 | 0.51 | 0.40 | 0.24 | 0.19 | 0.18 | 0.10 | 0.01 |
| Descriptive statistics of traits among core subset | | | | | | | | | | | | | | | |
| Min | 42.4 | 11.0 | 20.0 | 0.10 | 3.73 | 3.00 | 3.50 | 0.30 | 3.27 | 0.30 | 1.07 | 0.10 | 3.90 | 0.10 | 2.67 |
| Max | 110.0 | 265.0 | 435.0 | 0.34 | 14.07 | 13.00 | 29.00 | 1.03 | 9.00 | 1.03 | 9.57 | 0.45 | 12.00 | 0.45 | 11.95 |
| average | 75.0 | 83.6 | 136.9 | 0.19 | 8.47 | 5.71 | 14.31 | 0.59 | 6.04 | 0.59 | 5.90 | 0.32 | 7.27 | 0.32 | 5.52 |
| SD | 16.03 | 59.24 | 100.1 | 0.05 | 2.52 | 2.06 | 5.52 | 0.17 | 1.28 | 0.17 | 1.94 | 0.08 | 1.58 | 0.07 | 1.72 |
| Kurtosis | 0.13 | 1.69 | 1.97 | 2.82 | -0.41 | 3.53 | 1.20 | 0.75 | 0.49 | 0.58 | 0.23 | 0.76 | 1.75 | 1.44 | 6.42 |
| Skewness | 0.297991 | 1.28 | 1.26 | 0.44 | -0.13 | 1.83 | 0.72 | 0.25 | 0.56 | 0.26 | -0.26 | -0.16 | 0.36 | -0.53 | 2.11 |
| t test* | 0.406 | 0.190 | 0.262 | 0.425 | 0.375 | 0.195 | 0.427 | 0.337 | 0.369 | 0.381 | 0.045 | 0.021 | 0.341 | 0.025 | 0.349 |

C1 = length of main tiller (cm); C2 = number of tillers/tussock; C3 = fresh weight/tussock (g); C4 = tiller internode diameter (cm); C5 = 4th inter-nodal length (cm); C6 = number of nodes on main tiller; C7 = 4th leaf blade length (cm); C8 = 4th leaf blade width (cm); C9 = 4th leaf sheath length (cm); C10 = 4th leaf sheath width (cm); C11 = flag leaf blade length (cm); C12 = flag leaf blade width (cm); C13 = flag leaf sheath length (cm); C14 = flag leaf sheath width (cm); C15 = inflorescence length (cm); EV = Eigen Values; VP = variance proportion; CP = cumulative proportion; * t test core subset vs. all accessions (shows P values).

yield (-0.076). A difference of almost 3 times was noted for tiller internode diameter, which ranged from 1 to 3 mm. Internodal length between the 3rd and 4th nodes varied from 3 to 14 cm with an average of 8.32 cm. Number of nodes per tiller varied from 3 to 13. Leaf blade and leaf sheath lengths varied from 3 to 29 cm and 3 to 9 cm, respectively. Leaf blade and leaf sheath widths both varied from 0.3 to 1 cm. Such variation was also noted for flag leaf length and width. Flag leaf blade length differed among accessions from 1 to 10 cm, whereas flag leaf sheath length differed from 3 to 12 cm. Width of flag leaf blade and sheath varied from 0.1 to 0.6 cm and 0.1 to 0.35 cm, respectively. Inflorescence length also varied significantly, i.e. 2 to 11 cm.

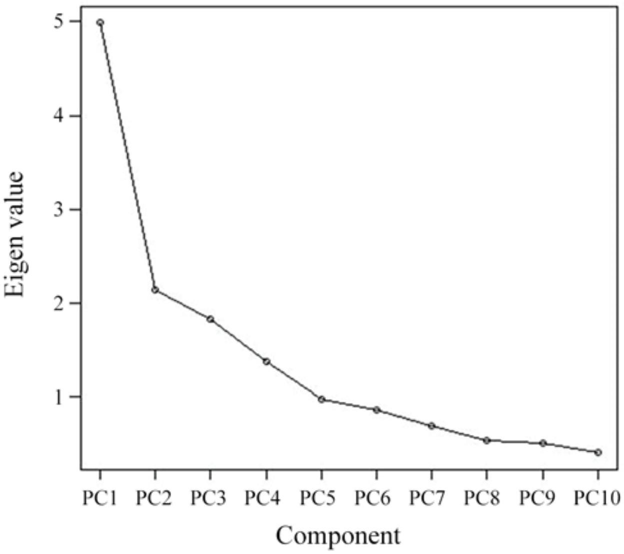

**Figure 2.** Scree plot of Heteropogon contortus accessions.

Visual observation of non-metric traits revealed that 97 accessions were poor to very poor in growth, whereas 72 accessions were good to very good (Table 5). As regards plant habit, 157 accessions were decumbent, 76 were erect and only 2 were prostrate. Tiller internode color varied from violet to green with some mixed shades. Greenish-violet tiller was predominant with 114 accessions, whereas violet and light violet figured in 54 accessions. Sixty-seven accessions had light green tillers. Nodes were mostly greenish-violet to dark violet, except in 16 accessions, which possessed green nodes. Nodes were predominantly non-hairy and only 60 accessions possessed scant hair on nodes. Leaf surface of 164 accessions was glabrous, while 71 accessions had medium or scant hairs. Light green and green were the most dominant leaf blade colors with 170 and 53 accessions, respectively. Two accessions were blue-green and 6 accessions had greenish-blue leaf blades. Green leaf sheath color was also dominant with 171 accessions being green or light green. Sixty-four accessions were noted with greenish-violet leaf sheaths. Anthocyanin coloration on leaf surfaces was less common than on leaf sheath surfaces and nodes. Spike color was green among all accessions but varied in intensity. Except for 2 accessions which possessed soft awns, accessions had hard awns. The soft-awned accessions were short-lived annual types. Ligule length ranged from 0.5 to 1 mm among accessions, with the majority being 1 mm.

One of the important nutritional parameters, crude protein concentration, varied from 2 to 10% with an average of 5.7%, whereas IVDMD varied from 31 to 59% with an average of 46.2% (Table 6). Variation in fiber concentrations ranged from 74 to 87% (average

**Table 5.** Variation for non-metric traits and the ligule length in 235 *Heteropogon contortus* accessions.

| Growth | | Habit | | Leaf hairiness | | Leaf blade color | | Leaf sheath color | |
|---|---|---|---|---|---|---|---|---|---|
| Good | 39 | Decumbent | 157 | Glabrous | 164 | BG | 2 | Green | 101 |
| Medium | 66 | Erect | 76 | Light hairy | 65 | G | 53 | GV | 64 |
| Poor | 61 | Prostrate | 2 | Hairy | 6 | GV | 4 | LG | 70 |
| Very Good | 33 | | | | | GB | 6 | | |
| Very Poor | 36 | | | | | LG | 170 | | |
| Node color | | Node hairiness | | Tiller internode color | | Anthocyanin coloration on leaf blade | | Anthocyanin coloration on leaf sheath | |
| DV | 60 | LH | 60 | GV | 114 | Absent | 151 | Absent | 88 |
| G | 16 | NH | 175 | LG | 67 | Medium | 46 | Strong | 30 |
| GV | 104 | | | LV | 14 | Strong | 29 | Weak | 117 |
| V | 55 | | | V | 40 | Weak | 9 | | |
| Node anthocyanin coloration | | Spike color | | Awn pubescence distribution | | Awn stature | | Ligule length (mm) | |
| Absent | 2 | G | 185 | 50% | 203 | Hard | 233 | 0.05 | 42 |
| DV | 72 | GV | 30 | 100% | 32 | Soft | 2 | 0.1 | 193 |
| LV | 32 | LG | 20 | | | | | | |
| V | 129 | | | | | | | | |

BG = bluish-green; G = green; GV = greenish-violet; LG = light green; LV = light violet; V = violet; GB = greenish-blue; DV = dark violet; LH = Light hairy; NH = Non-hairy; Number against traits are number of accessions.

**Table 6.** Nutritional parameters of 167 accessions of *Heteropogon contortus*.

| | Ash % | OM % | CP % | NDF % | ADF % | Hemi-cellulose % | Cellulose % | Lignin % | IVDMD[1] % |
|---|---|---|---|---|---|---|---|---|---|
| Among accessions | | | | | | | | | |
| Min | 5.35 | 87.9 | 2.14 | 74.4 | 38.2 | 23.72 | 22.9 | 2.70 | 31.5 |
| Max | 12.10 | 94.7 | 10.10 | 87.1 | 59.0 | 44.89 | 51.1 | 21.41 | 59.9 |
| Average | 8.58 | 91.4 | 5.67 | 81.7 | 48.0 | 33.70 | 36.9 | 7.39 | 46.2 |
| Mean values among clusters | | | | | | | | | |
| Cluster 1 | 8.60 | 91.4 | 5.93 | 81.8 | 47.1 | 34.7 | 36.2 | 7.18 | 46.3 |
| Cluster 2 | 7.67 | 92.3 | 5.06 | 81.7 | 47.1 | 34.6 | 36.5 | 6.49 | 44.9 |
| Cluster 3 | 8.69 | 91.3 | 5.52 | 81.9 | 47.6 | 34.3 | 36.2 | 7.76 | 46.0 |
| Cluster 4 | 8.48 | 91.5 | 5.76 | 81.6 | 48.7 | 32.8 | 37.9 | 7.20 | 46.4 |
| Cluster 5 | 9.00 | 91.0 | 5.57 | 81.4 | 47.0 | 34.4 | 35.7 | 6.48 | 51.8 |
| Cluster 6 | 9.56 | 90.4 | 5.37 | 78.9 | 47.6 | 31.3 | 33.9 | 7.57 | 48.6 |
| Among core subset accessions | | | | | | | | | |
| Min | 5.87 | 89.45 | 3.81 | 78.6 | 41.8 | 28.0 | 31.3 | 2.70 | 36.0 |
| Max | 10.55 | 94.13 | 7.79 | 85.7 | 52.8 | 39.1 | 41.7 | 8.04 | 52.5 |
| Average | 8.67 | 91.33 | 5.69 | 81.7 | 47.6 | 34.1 | 36.4 | 6.58 | 46.6 |
| t test[2] | 0.375 | 0.375 | 0.462 | 0.470 | 0.230 | 0.264 | 0.214 | 0.004 | 0.381 |

OM = organic matter; CP = crude protein concentration; NDF = neutral detergent fiber concentration; ADF = acid detergent fiber concentration; Cell = cellulose; Lig = lignin; IVDMD = *in vitro* dry matter digestibility.

[1] 81 accessions analyzed.

[2] t test core subset vs. all accessions (shows P values).

**Table 7.** Core subset of *Heteropogon contortus* germplasm.

| Cluster | Original size | Log value | Proportion of log value | No. of accessions to be selected | No. of second-level cluster formed | No. of accessions selected from each cluster | Accession number (as per SN in Table 1) |
|---|---|---|---|---|---|---|---|
| 1 | 30 | 1.477 | 0.21 | 8 | 8 | 8 | 5, 13, 46, 53, 90, 115, 153, 214 |
| 2 | 3 | 0.477 | 0.07 | 2 | 2 | 2 | 74, 231 |
| 3 | 101 | 2.004 | 0.29 | 10 | 10 | 10 | 9, 22, 35, 85, 91, 105, 126, 147, 207, 222 |
| 4 | 94 | 1.973 | 0.28 | 10 | 10 | 10 | 20, 23, 54, 66, 101, 121, 158, 160, 184, 229 |
| 5 | 5 | 0.699 | 0.10 | 3 | 3 | 3 | 17, 122, 218 |
| 6 | 2 | 0.301 | 0.04 | 2 | 1 | 2 | 73, 123 |
| Total | 235 | 6.931 | | 35 | | 35 | |

81.7%) for NDF and 38 to 58% (average 48.0%) for ADF. Differences for lignin were much greater and ranged from 2.7 to 21.4%. Ash concentration ranged from 5 to 12% and organic matter from 87 to 94%. Accessions showed variation for hemicellulose and cellulose, which varied from 23 to 44% and 22 to 51%, respectively.

A subset of 35 accessions was identified as a core germplasm set, representing 15% of the total germplasm evaluated (Table 7). This subset represented 8, 2, 10, 10, 3 and 1 accessions from Clusters 1 to 6, respectively. Almost all variability was captured in the core subset (Tables 4 and 6). Student's t-test for various morphological traits of the core subset against the total accessions showed non-significant variation, except for flag leaf blade length and width and flag leaf sheath width (Table 4). Similarly, t-test for various nutritional parameters of the core subset against the total accessions showed non-significant variation, except for lignin concentration (Table 6).

## Discussion

Analysis of data recorded on various morphological quantitative metric and non-metric traits, as well as nutritive parameters established high variability among *Heteropogon contortus* accessions. The germplasm represented tropical semi-arid climates (126 accessions from Chhattisgarh, Madhya Pradesh and Maharashtra), tropical arid climate (29 accessions from Rajasthan), tropical subhumid climate (9 accessions from Tamil Nadu, Karnataka and Kerala), subtropical semi-arid climate (63 accessions from Uttar Pradesh) and subtemperate to temperate climate (8 accessions from Himachal Pradesh, Jammu and Kashmir). A high degree of genetic variation was also noted for non-metric traits, growth and habit along with nutritional parameters. Clustering of accessions collected from different places indicated that these accessions either originated from a common source or moved from one place to other, resulting in lesser inter-population differences. Grouping of material based on statistical numerical procedures helps in understanding of variability, which further becomes the basis for identification of core germplasm, which can be exploited in breeding programs. Development of a core subset is effective when it is truly representative of the variation present in the germplasm collection. A comparison of the data shows that almost all the variability has been captured in the core subset.

The study established high genetic variability and correlation of some traits with biomass production. Tiller number, high nodal number and longer leaf in *H. contortus* were reported to be associated with forage yield (Roy 2004). Morphological traits like plant height, number of tillers and leaf length were also reported to be associated with forage yield and considered as important traits in constructing selection criteria for forage yield in the perennial grass *Sehima nervosum* (Roy et al. 1999). Earlier studies involving tropical perennial grasses indicated a wide range of diversity for different characters and the clustering pattern was observed to be independent of their geographical distribution in *Dichanthium* (Agarwal et al. 1999; Chauhan et al. 2007), *Sehima nervosum* (Roy et al. 1999), *H, contortus* (Roy 2004; Bhat and Roy 2007; 2014) and Guinea grass (Jain et al. 2003a, 2003b, 2006; Roy et al. 2020). The annual accessions, with soft awns, were quite low in biomass. In fact, these accessions were short-lived perennials and could not survive in the harsh climate of Jhansi, India (max temp 45 °C during May and June). Such annual types have been described from India earlier (Soromessa 2011). In earlier studies, evaluation of accessions of *H. contortus* collected from different parts of India revealed high values for heritability, genetic advance and genotypic and phenotypic coefficients of variation for tiller number/tussock, green fodder yield and dry matter yield (Roy 2004), and were considered to be useful for selection of accessions. An isozymic study on *H. contortus* accessions indicated high genetic intra-species diversity; however, clustering could not be correlated with the geographical origin of the accessions (Bhat and Roy 2014).

The present study identified the wide range of genetic variation among the germplasm set evaluated, which is in congruence with earlier reports of high genetic variation. The presence of genetic variability in the germplasm collection of this apomictic grass can be attributed to recombinations taking place owing to residual sexuality among some plants. The grass is an aposporous apomict with 2n = 20, 40, 44, 60 and 80 (Fedorov 1974; Srivastava and Purnima 1990). Although it is primarily apomictic in nature, it shows high genetic variability in subhumid dry regions of south India, probably due to the presence of residual sexuality in a few accessions. Genetic diversity in the species has been reported by Carino and Daehler (1999) utilizing molecular studies within and among Hawaiian populations of the species. Roy (2004) and Bhat and Roy (2007; 2014) have also reported high genetic variability among germplasm based on morphological and isozyme studies. A considerable amount of localized variation was reported in the early botanical literature because of occasional sexual reproduction in *H. contortus* (Soromessa 2011). The lack of uniformity among individuals in Hawaiian populations of this apomictic grass, based on RAPD studies, indicated frequent sexual reproduction (Carino and Daehler 1999). Diverse forms collected from the same location (Bhat and Roy 2007) also indicate presence of some recombinations taking place through residual sexuality among some plants.

Other factors could also be responsible for such variation. Introduction of germplasm from other countries over different time periods is one potential source of variation, but there are no documented reports of this occurring. However, dispersal of seed through other biotic and abiotic sources cannot be ruled out. One plausible explanation of seed dispersal may be its hard awns, which get stuck in animal or human bodies or coats.

The collections represent different agro-ecological zones of the country and variation was found within local populations as well as between populations, which indicates a distinct possibility of sexual reproduction taking place, unless the species was introduced into India from more than one source. Further domestication and recombination might have contributed more to the variability. The presence of among-population differentiation but lack of between-island differentiation was considered to indicate that *H. contortus* was an early Polynesian introduction to the Hawaiian Islands (Carino and Daehler 1999). Hence, further molecular studies involving Indian germplasm and germplasm from some other countries, particularly neighboring ones, may give some idea on possible movement of germplasm. The study will help researchers to focus future studies on this core subset in developing genetic improvement programmes.

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## Conflicts of interest

The authors declare that they have no conflict of interest.

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