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Collection and evaluation of genetic diversity in Dinanath grass (*Pennisetum pedicellatum* Trin.) for forage yield and leaf blight resistance

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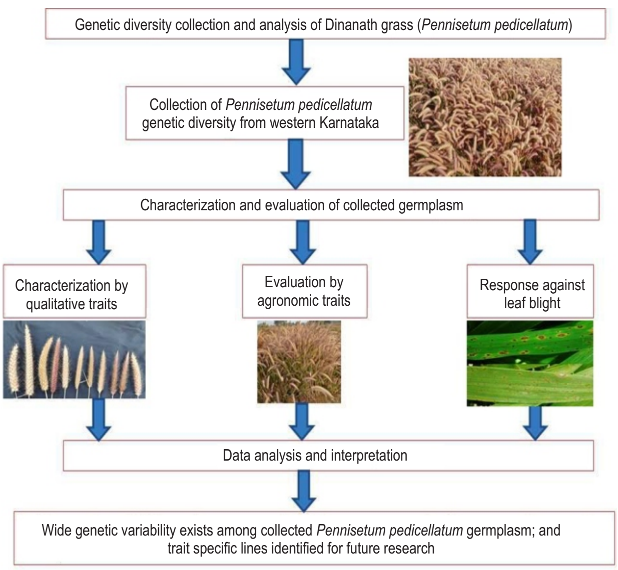
Abstract

Aim: Dinanath grass is a drought tolerant multipurpose species with high potential for quality fodder in tropical regions. In India, it is distributed in eastern and southern parts. The gap in collection and exploitation of genetic diversity exists in Dinanath grass. Therefore, genetic diversity of Dinanath grass was collected and evaluated for utilization in genetic improvement of *Pennisetum* species for future fodder security.

Methodology: A total 28 accessions of Dinanath grass were collected from western and southern regions of Karnataka. Collected accessions were grown under rainfed conditions and evaluated for six qualitative and ten quantitative traits and response to leaf blight reaction. The evaluated accessions were analyzed by using different univariate and multivariate statistical tools.

Results: Dinanath accessions were categorized as per qualitative traits. Quantitative traits exhibited low (7.43%) to high (62.88%) coefficient of variation. Dry matter yield showed positive association with plant height, leaf area and tillers per plant. Cluster analysis classified Dinanath accessions into four distinct groups. The first five principal components explained >85% of the total variation. Three accessions *viz.,* IG-15-26, IG-15-30, IG-15-4 were identified as resistant against leaf blight disease; and donor accessions for agronomic traits were selected.

Interpretation: The indigenous Dinanath grass collection from Western Karnataka has ample diversity with reference to qualitative and biomass contributing traits; and resistance against leaf bight disease. The information generated on collected germplasm will assist the researchers in designing the *Pennisetum* genetic improvement programme.



Key words: *Bipolaris*, Genetic diversity, Leaf blight, Multivariate analysis, Trait specific accessions

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Introduction

Dinanath grass (*Pennisetum pedicellatum* Trin.) belongs to the tribe Paniceae of family Poaceae and widely distributed in West Africa and India. In India, it is distributed in Andhra Pradesh, Bihar, Chhattisgarh, Jharkhand, Karnataka, Maharashtra, Odisha, West Bengal, Andaman & Nicobar islands (Nayar *et al.,* 2009; Upadhyaya *et al.,* 2014). It is probably spread from India to the South East Asia and Northern Australia (Schmelzer, 1997). Dinanath grass is an annual, quick growing, luscious, leafy, thin stemmed and grows well even on poor, eroded soils (Mukherjee *et al.,* 1982); and has very strong tolerance under severe drought conditions (Noitsakis *et al.,* 1994). Dinanath grass is having high importance in providing high quantum of quality forage for maintaining animal nutritional security under drought and marginal soil besides its uses in ornamental, soil erosion control, improvement in the physical and chemical properties of the soil (Kumar and Jena, 1996) and as a bio-energy crop (Kumar and Ghosh, 2018). Dinanath grass is widely used for animal feed as green fodder, hay and silage. However, its biomass productivity and forage quality largely depends upon stage of harvest (Asmare *et al.,* 2017; Tilahun *et al.,* 2017). Crop cutting at 60-65 days results in a high crude protein (9.06%), high crude fat (2.55%) and low crude fibre (28.95%) contents (Khan *et al.,* 1995). Dinanath grass is also rich in sodium, potassium, phosphorus and calcium has the potential to be used in alleviating macro and micro-nutrients deficiencies in animals (Mustapha *et al.,* 2018; Suleiman *et al.,* 2020).

Genetic diversity is the base for survival of species and crop improvement programmes. Genetic diversity in different crops were collected and evaluated for conservation and utilization in breeding programme (Singh *et al.,* 2014; Trivedi *et al.,* 2018). For utilization in breeding programme, diversity of forage grasses were also analyzed by using different molecular, agronomic and quality traits (Saxena and Chandra, 2011; Zeki *et al.,* 2009). *P. pedicellatum* though having multiple uses and is a wild relative of many cultivated species being contributing in form of donor for biotic and abiotic stress tolerance (Singh and Navi, 2000). But it has not received due attention from germplasm collectors. The diversity of *P. pedicellatum* distributed in different parts of the country is not represented in the collected genepool in International and national genebanks; and a geographical collection gaps are present mainly in south-western part of India (Upadhyaya *et al.,* 2014).

The continuous genetic erosion is a serious threat to the genetic variability accumulated over centuries, mainly by modern cultivation practices, concomitant natural catastrophes (droughts, floods, fire hazards etc.), human settlements, overgrazing etc. Climate change is likely to be an additional threat to agricultural biodiversity, increasing genetic erosion of landraces and threatening wild species, including crop wild relatives (Jarvis *et al.,* 2008). Further, characterization and evaluation is the key to assess the potential and actual value of collected germplasm. Therefore, in order to prevent genetic erosion, Dinanath grass diversity was collected in wild habitats from unexplored areas of Karnataka and evaluated for the utilization in future *Pennisetum* improvement programme through sustainable ways.

Materials and Methods

Collection of genetic diversity: Four districts of western and southern Karnataka, India *viz.,* Belgaum, Dharwad, Uttara Kannada and Bengaluru urban were explored. Passport characters e.g. village, block, district, latitude, longitude, altitude and habitat were recorded at the collection sites. The samples were mainly collected in the form of inflorescence, seeds and rooted slips. Random sampling method was followed for collecting single panicle from at least 50 plants, with 95 percent certainty, all the alleles at a random locus occurring in the target population with frequency greater than 0.05 (Marshall and Brown, 1975).

Evaluation of collected germplasm

Location of experiment: The experiment was conducted during 2018-2019 at the Central Research Farm of the ICAR-Indian Grassland and Fodder Research Institute, Jhansi, India (latitude 25°31' N, longitude 78°32' E and altitude 237 m above msl). The experimental site is characterized by a semi-arid climate with extreme temperatures during summer (43-46°C) and winter (as low as 2°C). The soil was deep, moderately well drained, and brown to dark grayish brown with fine loamy texture. Seeds of collected germplasm (28 accessions) and one check (cv. Bundel Dinanath-2) were grown in pots and after reaching seedling height around 30cm they were then transplanted in the field. Each accession was planted in a plot size of 3m×1m in CRB design with two replications. Line to line and plant to plant distance was maintained at 50 × 50 cm with 1m spacing between two plots. The crop was grown under rainfed conditions and managed following the standard agricultural practices.

Data recording and statistical analysis: Meteorological observations *viz.,* weekly mean temperature (°C), rainfall (mm), relative humidity (%) and evaporation (mm) were recorded during the crop growth period. Data was recorded on six qualitative traits including early plant vigour, plant growth habit, culm colour, branching pattern, leaf hairiness, spikelet colour; and ten quantitative traits *i.e.,* plant height (cm), days to maturity, flag leaf length (cm), flag leaf width (cm), penultimate^{-1} leaf length (cm), penultimate^{-1} leaf width (cm), peduncle length (cm), panicle length (cm), number of tillers per plant, dry matter yield per plant (g). Data on qualitative traits were recorded on plot basis and on quantitative traits were recorded on five representative plants.

The accessions were classified based on the descriptor states of qualitative traits and presented through bar graph. The mean data of quantitative traits were analyzed by univariate statistics e.g. means, ranges and variance; and multivariate analysis including cluster, correlation and principal component. The correlations among the traits were based on the Pearson

Correlation Coefficients. Clustering of accessions was carried out using 'Cluster' package of SAS statistical software with squared euclidean distance. Dendrogram was constructed on the basis of fusion level to examine similarities in the pattern of performance among accessions. Data of all characters were standardized to a mean of zero and variance of one and Principal Component Analysis (PCA) was done to investigate the importance of different traits in explaining multivariate polymorphism in Dinanath grass accessions.

Leaf blight (*Bipolaris sorokiniana*) severity was recorded under natural epiphytotic conditions. The accessions which showed resistant and moderately resistant under natural conditions were further screened *in-vitro* for confirmation. The screening was carried out in petri dishes by inoculating detached leaves with blight spores using the spot inoculation method. Disease severity was recorded through visual estimation using disease scale (0-4) given by Takahashi *et al.* (2009) and based on disease reaction the accessions were classified as highly resistant (HR), resistant (R), moderately resistant (MR), susceptible (S) and highly susceptible (HS).

Results and Discussion

Twenty eight accessions of Dinanath grass were collected from diverse ecological niches of four western and southern Karnataka districts *viz.,* Belgaum, Dharwad, Uttar Kannada and Bangaluru urban. During evaluation of collected germplasm the meteorological observations including weekly temperature, rainfall, relative humidity and evaporation were recorded. The average minimum temperature varied from 4.8-22.9°C, maximum temperature 21.9-35.8°C, relative humidity (RH I) 75-94%, RH II 36-85 and evaporation 2.3-5.4 mm. The total precipitation during crop season was 191.8 mm and most of the precipitation (175.6 mm) occurs during the first week of transplanting of crop and remaining (16.2 mm) occur in third week and after that no rainfall occur and the crop survived on residual soil moisture. Being a C4 plant Dinanath grass is more efficient in utilization of water and nitrogen. The present study also support that Dinanath grass is drought resistant and can grow under prevailing minimal rainfall condition as reported earlier by Noitsakis *et al.* (1994).

Analysis of morphological diversity revealed that mostly accessions showed good early plant vigour, purple type culm, medium branching pattern, leaf hairiness and purple type spikelets. Distribution of Dinanath accessions as per descriptor states are mentioned in Fig. 1. Out of the total, one accession DSN/MK-92 with purple culm and spikelet colour can be grown as morphological marker and also for ornamental value. Quantitative traits exhibit low (7.43%) to high (51.99%) value of coefficient of variation (CV). Plant height and dry matter yield per plant are important traits on fodder point of view. Dry matter yield per plant varied from 11.4g to 264.4g with 51.99% CV and height of the plant varied from 47.70cm to 106.6cm with a mean of 79.88cm and high CV (21.44 %) suggests significant variability among the accessions. Days to maturity varies from 98 days to 127 days and showed low CV, indicates less variability among evaluated

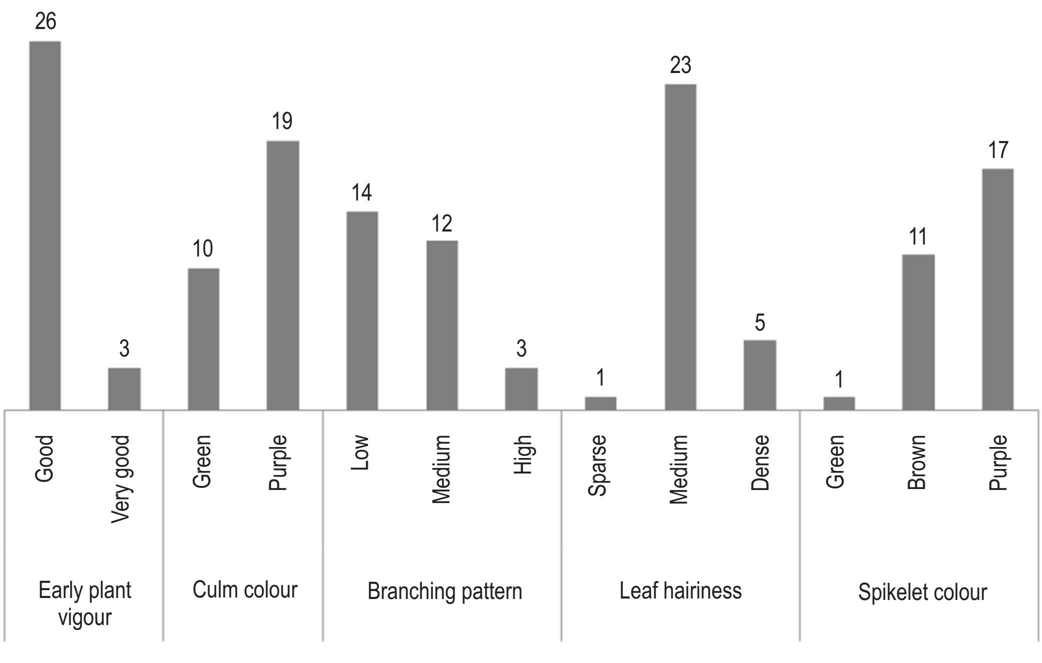

Fig. 1: Distribution of *Pennisetum pedicellatum* accessions as per expression of descriptor states of qualitative traits.

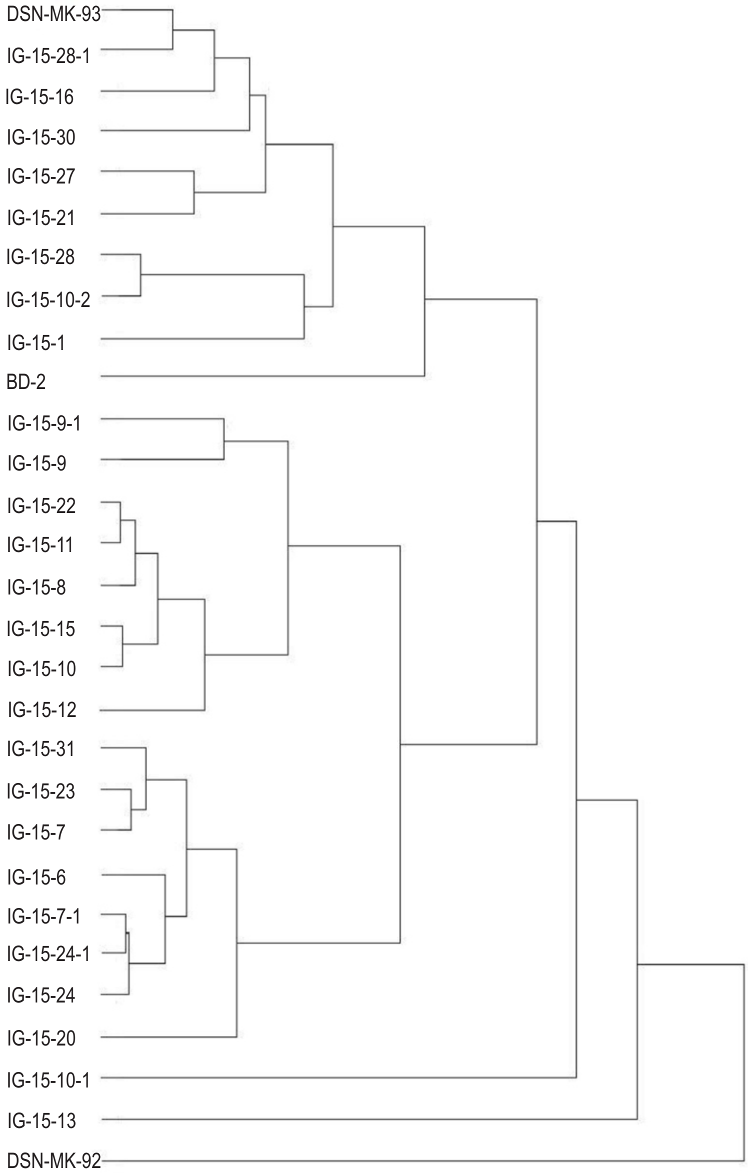

Fig. 2: Distribution of Dinanath grass accessions into different clusters based on 10 agronomic traits.

accessions. Moderate variability was recorded for flag leaf length, penultimate^{-1} leaf length, peduncle length and panicle length whereas high CV were recorded for flag leaf width, penultimate^{-1} leaf width, number of tillers per plant, plant height and dry matter yield per plant (Table 1). High variability in biomass yield and its contributing traits were also reported in global Dinanath grass collection by Upadhyaya *et al.* (2014) and Mishra *et al.* (2009). High variability recorded among biomass contributing traits suggest further scope of improvement through new breeding tools. However, further exploration should be planned to incorporate more variability in days to maturity and qualitative traits where low variability was observed in the present study.

Correlation coefficients analysis indicates mutual relationship between various metric traits and determines the component characters which are associated with biomass yield. Out of total 45 associations among 10 agronomic traits, 7 associations were significant (p<.05) either in positive or negative direction (Table 2). Panicle length was strongly correlated with peduncle length (r=0.78) followed by penultimate^{-1} leaf length with panicle length (r=0.66) and peduncle length (r=0.60); and flag leaf length with flag leaf width (0.50). Tillers per plant and penultimate^{-1} leaf length showed negative correlation (r=-0.71) followed by panicle length with tillers per plant (r=-0.47). Dry matter yield per plant (DMY) showed positive association with all studied traits;

Table 1: Variation in agronomic traits among 29 Dinanath grass accessions evaluated in Central India

Traits	Mean ± SE	Variance	Kurtosis	Skewness	Range	CV%
Plant height (cm)	79.88± 3.18	293.55	-0.844	-0.24	47.70-106.6	21.44
Days to maturity	115.55±1.59	73.68	-1.04	-0.38	98-127	7.43
Flag leaf length (cm)	6.85±0.24	1.72	-0.59	0.23	4.50-9.44	19.16
Flag leaf width(cm)	0.30±0.02	0.01	0.94	1.18	0.16-0.64	39.87
Penultimate^{-1} leaf length (cm)	11.52±0.30	2.61	3.98	-1.5	5.90-13.92	14.02
Penultimate^{-1} leaf width (cm)	0.63±0.03	0.03	-0.24	-0.09	0.24-0.98	27.57
Peduncle length (cm)	29.64±0.82	19.56	1.27	-0.84	17.50-37.50	14.92
Panicle length (cm)	13.89±0.41	4.8	1.17	-0.08	8.14-19.18	15.78
Tillers per plant	49.3±4.32	542.29	7.33	2.33	19.50-139.40	47.20
Dry matter yield per plant (g)	136±13.21	5065.83	-0.84	0.00	11.4-264.4	51.99

Table 2: Correlation coefficient among quantitative traits recorded on 29 accessions of Dinanath grass

Traits!	PHT	DTM	FLL	FLW	PLL	PLW	PDL	PNL	TPP
DTM	0.07								
FLL	0.31	-0.05							
FLW	-0.01	-0.12	0.50**						
PLL	0.14	0.23	0.23	-0.07					
PLW	-0.18	0.16	0.27	0.36	0.14				
PDL	0.03	0.27	0.18	0.05	0.60**	-0.01			
PNL	0.18	0.20	0.03	-0.13	0.66**	-0.21	0.78**		
TPP	-0.15	-0.16	-0.25	0.20	-0.71**	-0.11	-0.27	-0.47*	
DMY	0.42*	0.18	0.16	0.02	0.23	0.19	0.15	0.22	0.29

!PHT-Plant height, DTM-Days to maturity, FLL-Flag leaf length, FLW-Flag leaf width, PLL-Penultimate^{-1} leaf length, PLW-Penultimate^{-1} leaf width, PDL-Peduncle length, PNL-Panicle length, TPP-Tillers per plant, DMY-Dry matter yield per plant.
*=significant at 5% probability level and **=highly significant at 1% probability level

maximum value of association was recorded with plant height (r=0.42), followed by tillers per plant, penultimate^{-1} leaf length, panicle length, peduncle length, penultimate^{-1} leaf width and days to maturity. The positive association of DMY with agronomic traits (plant height, tillers per plant and leaf length) may result from better competition for radiant energy with extended days to harvest. A similar correlation was also observed in other studies (Asmare *et al.,* 2017; Gadisha *et al.,* 2019). The principal component (PC) analysis was effective in that the first five PC accounted for 85.37% of the total variation among the 29 germplasm accessions of Dinanath grass (Table 3). Characters with high variability are expected to provide high level of gene transfer during breeding programs. Accordingly, in the first PC, penultimate^{-1} leaf length, panicle length and peduncle length were the most important traits contributing to variation that explained 29.69% of the total variance. In the second PC, which describe 18.97% of the total variance originated mainly from penultimate^{-1} leaf width, flag leaf length and flag leaf width. Similarly, DMY, flag leaf width, leaflet length constituted a large part of total variance explained by the third PC. In the fourth PC, which describe 11.59% of the total variance, originated mainly from penultimate^{-1} leaf width and peduncle length. The fifth PC describes 9.13% mainly due to days to maturity, tillers per plant and plant height.

The cluster analysis placed 29 Dinanath grass accessions into five clusters (Fig. 2). Cluster one includes 10 accessions, cluster two 16 accessions while cluster third, fourth and fifth with one accession each. Dinanath accessions falling in different clusters were most diverged than others viz., DSN MK-93, BD-2, IG-15-9-1, IG-15-20, DSN-MK-92 and check variety Bundel Dinanath-2. This indicated that these accessions are most suitable for utilization in future genetic improvement programme. Grouping of accessions showed that cluster I and cluster II pooled with maximum number of accessions which indicated similarities for phenotypic traits and genetic lineage. The reason for grouping of accessions of same geographic origin into different clusters might be due to the different genetic architecture resulted wide divergence in morphological features. The distribution of germplasm accessions in the study indicated that the geographical origin did not have any bearing on clustering pattern. Though, all genotypes were collected from Karnataka state but placed in different clusters, which indicated that the geographical distribution and genetic divergence did not follow the same trend. The genetic drift and selection could be the cause greater diversity within a geographical region. The results are in agreement with the previous findings of Upadhyaya *et al.* (2014) for similar reasons of diversity in Dinanath grass.

Table 3: Eigen values and Eigen vectors of the important principal components (PC) for variation among the 29 accessions of Dinanath grass

Traits	PC1	PC2	PC3	PC4	PC5
Plant height	0.154	-0.003	0.191	-0.753	0.343
Days to maturity	0.212	0.126	-0.425	0.091	0.715
Flag eaf length	0.175	0.460	0.390	-0.259	-0.025
Flag leaf width	-0.045	0.444	0.482	0.190	0.165
Penultimate^{-1} leaf length	0.517	0.078	-0.090	0.027	-0.236
Penultimate^{-1} leaf width	0.024	0.558	-0.070	0.328	-0.079
Peduncle length	0.456	-0.116	0.194	0.351	0.230
Panicle length	0.493	-0.266	0.117	0.119	0.056
Tillers per plant	-0.424	-0.104	0.198	0.212	0.475
Dry matter yield per plant	0.000	-0.403	0.543	0.173	-0.006
Eigen value	2.97	1.90	1.60	1.16	0.91
Percent of variance explained	29.69	18.97	15.99	11.59	9.13
Cumulative percent of total variance explained	29.69	48.65	64.65	76.24	85.37

Table 4: Selection of superior Dinanath grass accessions for agronomic traits

Traits	Value	Genotype (trait value)
Plant height	>100cm	IG-15-12 (100.2), IG-15-1 (101.8), IG-15-15 (102.3), IG-15-10 (103.2), IG-15-27 (106.6)
Days to maturity	≤108 days	DSN-MK-92 (98), Bundel Dinanath-2 (100), IG-15-1 (103), IG-15-28 (106), IG-15-22 (108)
Days to maturity	≥124 days	IG-15-10-1 (124), IG-15-30 (125), IG-15-27 (126), IG-15-7-1 (127), IG-15-7 (127)
Flag leaf	Length (≥8.4cm)	IG-15-27 (8.4), IG-15-21 (8.8), IG-15-16 (8.8), IG-15-13 (9.0), IG-15-28 (9.4)
	Width (≥0.4cm)	IG-15-27 (0.4), IG-15-28 (0.5), IG-15-28-1 (0.5), IG-15-30 (0.6), IG-15-1 (0.6)
Penultimate^{-1} leaf	Length (≥13cm)	IG-15-11 (13.0), IG-15-1 (13.0), IG-15-8 (13.4), IG-15-23 (13.6), IG-15-6 (13.9)
	Width (≥0.8cm)	IG-15-28-1 (0.8), IG-15-7 (0.8), IG-15-31 (0.9), IG-15-23 (0.9), IG-15-28 (1.0)
Panicle length	Large (≥15.8cm)	IG-15-11 (15.8), IG-15-9 (16.8), IG-15-22 (16.8), IG-15-8 (17.1), IG-15-9-1 (19.2)
Tillers per plant	≥69	IG-15-30 (69.0), IG-15-9 (72.6), IG-15-16 (74.0), IG-15-20 (90.3), DSN-MK-92 (139.4)
Dry biomass per plant	≥212.0gms	IG-15-12 (264.4), IG-15-15 (254), IG-15-9 (244), DSN-MK-93 (218.8), IG-15-16 (212)
Spot blotch reaction	Resistant	IG-15-26, IG-15-30, 1G-15-4
	Susceptible	IG-15-11, IG-15-3

Since the use of resistant varieties is considered to be the best approach for disease management in forage crops, therefore, the collected accessions along with Bundel Dinanath-2 cultivar were screened against *Bipolaris sorokiniana*. Among 29 accessions screened, three were resistant (IG-15-26, IG-15-30 and 1G-15-4), four moderately resistant (IG-15-1, IG-15-3, IG-15-11 and IG-15-27), seventeen susceptible and five highly susceptible in natural field conditions. Resistant and moderately resistant accessions were further verified *in-vitro*. The response of resistant accessions was similar to natural conditions but response of moderately resistant accessions differed under laboratory condition. Out of four moderately resistant accessions, two showed similar reaction and two (IG-15-3, IG-15-11) showed highly susceptible reaction. Our result is in accordance with Ojha *et al.* (2017) who showed that the disease response of wheat genotypes to leaf blotch pathogen varied between natural and artificial screening. Artificial screening verifies the results of natural screening (Chand *et al.,* 2008), thus artificial screening of genotypes is very important to confirm field resistance for accuracy. *B. sorokiniana* affects wide variety of grasses and cereals, and resistant donor against this disease has been identified in wheat and barley (Kumar *et al.,* 2019; Singh *et al.,* 2014). The distinct resistance identified in these accessions can be used as valuable source in breeding for spot blight disease in Dinanath grass and related species. Donor accessions were identified for agronomic traits and reaction to leaf blight disease (Table 4). Genotypes *viz.,* IG-15-10, IG-15-9 and IG-15-15 having good biomass yield, tall height, medium maturity and moderate tolerance to leaf blight disease needs to be tested for biomass production potential at multi-location for release as variety. High biomass producing accessions of Dinanath grass were also identified by Hidosa *et al.* (2020); Gadisha *et al.* (2019) and Yirgu *et al.* (2017). Donors for various traits in different crops were identified for enhance utilization of conserved germplasm in breeding programme (Kaur *et al.,* 2018; Singh *et al.,* 2014). Dinanath grass being a crop wild relative of Pearl millet and other cultivated *Pennisetum* species, the identified donor accessions could be utilized for transfer of useful novel genes like resistance against blight disease, tillering capacity and drought tolerance by exploiting new molecular breeding tools. Promising accessions identified for high dry matter yield and resistant to leaf blight disease will help in designing Dinanath grass improvement program and thus will also boost up fodder production and productivity in marginal land of low rainfall areas.

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Add-on Information

Authors' contribution: T. Singh: Contributed to the design and implementation of the research, germplasm collection, data recording and analysis, drafted and final the manuscript; **S.N. Dheeravathu:** Involved in germplasm collection and data recording; **N. Dikshit:** Processed the experimental data, data recording, performed the analysis and contributed to the final manuscript; **N. Manjunatha:** Performed disease analysis; **G. Sahay:** Aided in interpreting the results.

Research content: The research content is original and has not been published elsewhere.

Ethical approval: Not Applicable

Conflict of interest: The authors declare that there is no conflict of interest.

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