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Research Article

DUS characterization and diversity assessment in pearl millet inbreds

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Abstract
Plant morphological characters are universally undisputed descriptors applied for testing Distinctness, Uniformity and Stability (DUS) of crop varieties. In the present study, 49 inbred lines of pearl millet evaluated for 29 morphological traits using DUS descriptors revealed significant variation among the inbreds for different characters. Most of the qualitative characters revealed considerable variation except for the traits like spike exertion, node pubescence, inter node pigmentation, leaf sheath pubescence, anther color, glume pigmentation and plant growth habit. Large variation among inbreds were found for the quantitative traits like days to 50% flowering (40.33 - 58.55 days), leaf blade length (33.53 -72.13 cm), spike length (13.60 - 24.60 cm), number of productive tillers/plant (1.60 - 9.57), plant height (115.60 - 160.87 cm), 1000 grain weight (4.35 - 14.80 g), dry fodder yield/plant (57.74-84.37 g), green fodder yield/plant (192.34-517.53 g) and grain yield/plant (21.20 - 48.47 g). Dendrogram constructed on the basis of established quantitative characters among the individuals delineated 49 inbreds into six major clusters indicating ample diversity. The comprehensive morphological characterization of the lines with enormous diversity will facilitate their selection during pearl millet breeding programme and also provide documentation to protect them from any possible infringement.

Key words
DUS test, pearl millet, plant variety protection, dendrogram and cluster analysis

Introduction

Pearl millet (*Pennisetum glaucum* (L.) R. Br) is a diploid, photosynthetically efficient C_4 monocot belonging to family *poaceae* and genus *Pennisetum* having chromosome number 2n=14. It is a highly cross-pollinated crop with protogynous flowering nature which fulfills one of the essential biological requirements for hybrid development. Pearl millet is one of the major cereals grown, primarily for grain production, occupying more than 29 m ha in the arid and semi-arid tropical regions of Africa and Asia (Kannan *et. al*., 2014). India is the largest producer of pearl millet in Asia and occupies 7.95 mha area with production and productivity of 8.79 mt and 1106 kg/ha, respectively (Anonymous, 2014). In comparison to other cereals it exhibits resistant to drought and is mainly grown in *kharif* season in India. Pearl millet is a 'high energy' coarse cereal possessing high starch (70%), protein (10-12%) and 5-7% fat in dry grain. It is also rich in calcium and iron along with low fibre content.

The Government of India under the obligation of the TRIPS agreement passed the protection of Plant Varieties and Farmers' Right Act, 2001 (PPV & FR Act) to encourage public/private investment in research and development of new plant varieties by giving protection to the new plant genotypes against unauthorized multiplication of seeds or propagating materials for a specific period. PPV& FR Act, 2001 insists on DUS characterization of extant, farmers and new varieties and recommends the registration of varieties for atleast any one specific novel character. The new pearl millet genotypes will be protected under the PPV & FR Act after confirming DUS testing through the comparison of new and existing varieties in similar environment for a set of morphological characters. Researchers used morphological characters of plant, physical, physiological, biochemical, and molecular characterization of seed in crops like *Vicia faba* (Bond and Crofton, 2001), sorghum (Thangavel, 2003), lucerne (Senthilkumar, 2003), oat (Sumathi, 2007), rice (Eevera, 2003 and Maheshwaran, 2010), cotton (Ameena, 2009) and maize (Yadav *et al.,* 2010, Selvi *et al.,* 2013 and Mukesh *et al.,* 2014) for identification of genotypes. Characterization of these diverse inbreds for their morphological characteristics holds immense potential for their objective utilization in the breeding programme. Therefore, the present investigation was carried to characterize the pearl millet inbreds for selected DUS-characters, yield and yield attributing traits.

Materials and methods

Plant material and field trials : The experimental materials for the present study comprised of 49 (Table 1) stay green inbred lines (HSGR01 to HSGR44, H77/ 833 -2-202, H77/ 833 -2, H77/ 29 - 2, HBL–11, ICMR 01004) of pearl millet (*Pennisetum glaucum*).These inbreds were grown in a randomized block design (RBD) with three replications in a single row of 3 m length for each inbred, with spacing of 40 cm and 15 cm between-rows and plants, respectively during *kharif* season, 2014 at experimental research area of Department

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of Genetics & Plant breeding, CCS Haryana Agricultural University, Hisar. The standard cultural and agronomic practices were followed during crop growth.

Data collection and analysis: The observations on 13 quantitative traits were recorded for each genotype on five randomly selected plants from each replication, for the characters namely, leaf sheath length (cm), leaf blade length (cm), leaf blade width (cm), number of nodes/ plant, spike length (cm), spike girth at maximum point (cm), number of productive tillers/plant, plant height (cm) and 1000 grain weight (g). Days to 50% flowering (days) was recorded on row basis when the main panicles of 50% of the plants in the row had full stigma emergence. Data were also taken on 16 other qualitative traits. Among them, data for seedling color, panicle exertion, panicle tip sterility, node pubescence, node pigmentation, internode pigmentation, leaf sheath pubescence, anther color, glume pigmentation, presence/absence of bristles in panicle and bristles color was recorded on the basis of visual assessment of individual plants (or parts of plants) within a plot (VS), while for traits such as plant growth habit, panicle shape, panicle density, seed color and seed shape it was based on visual assessment of group of plants (or parts of plants) in a plot (VG). Three additional yield related characters namely dry fodder yield/plant (g), green fodder yield /plant (g) and grain yield/ plant (g) were also recorded after harvesting. Cluster analysis and Principal Coordinate Analysis were done using DARwin 6.0 software.

Results and discussion

Quantitative traits: The overall mean for 13 quantitative characters revealed that all the characters had sufficient variability among the 49 inbred lines (Table 2). Large variation were found for the traits like days to 50% flowering (40.33-58.33 days), leaf blade length (33.53-72.13 cm), spike length (13.60-24.60 cm), number of productive tillers/plant (1.60-9.57), plant height (115.60-160.87 cm), 1000 grain weight (4.35-14.80 g), dry fodder yield/plant (57.74-284.37 g), green fodder yield/plant (192.34-517.53 g) and grain yield/plant (21.20-48.47 g) in the present study. The wide range of variation observed for these characters offers an immense scope for the selection and evaluation of desirable inbreds for their further utilization as parental lines in pearl millet breeding programme. These significant differences could be attributed to the diverse genetic composition of the population comprising of dissimilar inbreds.

Dendrogram construction: In the present study, all the 49 inbred lines of pearl millet were significantly varied for all the characters under evaluation. These inbred lines were grouped into six clusters on the basis of 13 quantitative characters including yield and yield attributing characters (Figure 1), indicated the presence of considerable amount of genetic diversity. Clustering pattern revealed that, cluster 1 was the largest group consisting of 11 inbreds, followed by cluster 3 (10 inbreds), cluster 5 and cluster 6 (8 inbreds each), cluster 4 (7 inbreds) and cluster 2 (5 inbreds) where, the dissimilarity coefficient ranged between 0.44 and 2.85.

It was evident that mean value for grain yield/plant was the highest (44.72 g) in cluster 5 and lowest (25.18 g) in cluster 1 and cluster 2. Cluster 5 also revealed the highest mean value (5.95) for number of productive tillers/ plant, whereas, cluster 2 had the lowest mean value (2.73). For green fodder yield/plant cluster 5 and cluster 2 divulged the highest mean value (433.35 g) and lowest mean value (248.68 g), respectively. Cluster 5 showed the maximum mean value (11.38 g) for 1000 grain weight and cluster 2 showed the lowest mean value (7.01 g) for 1000 grain weight. Dry fodder yield/ plant had highest mean value in cluster 5 (192.31 g) and lowest mean value in cluster 4 (103.95 g). For days to 50% flowering highest mean value possessed by cluster 5 (50.62 days) and lowest mean value by cluster 4 (41.66 days). Cluster 1 had the highest (15.57 cm) and cluster 2 had the lowest mean value (13.56 cm) for leaf sheath length. For leaf blade length, cluster 4 (60.11 cm) and cluster 2 (42.46 cm) revealed the maximum and minimum mean value, respectively. Cluster 4 (3.13 cm) asserted the highest mean value and cluster 2 (1.94 cm) asserted the lowest mean value for leaf blade width. Plant height exhibited its highest and lowest mean values in cluster 5 (151.14 cm) and cluster 2 (120.03 cm), respectively. Number of nodes/plant was recorded highest in cluster 5 (9.18) and lowest in cluster 2 (6.61) for mean values. The cluster 5 and cluster 2 showed the maximum (22.38 cm) and minimum (17.04 cm) values of spike length, respectively. The mean value of spike girth was highest in cluster 3 (2.72) and lowest in cluster 2 (1.68), respectively. Cluster mean analysis (Table 3) indicated that cluster 5 had the highest mean value for nine characters. Therefore, cluster 5 was considered as most desirable for selecting inbreds. The result of cluster analysis was further supported by Principal Coordinate Analysis in which inbreds were distributed across four quadrangles and the distribution was highly consistent with the pedigree (Figure 2).

Inter & intra cluster distance (Figure 3) shows that maximum difference among the inbreds within the same cluster was shown by cluster 5 (4.403). This was followed by cluster 6 (3.956) and cluster 2 (3.796). Cluster 2 and 5 exhibited maximum divergence as indicated by their inter cluster

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distance of 7.999 followed the between clusters 4 and 5 (6.716). The lowest inter cluster distance was noticed between clusters 3 and 4 (4.197), followed by the clusters 1 and 2 (4.394) indicated that members of these clusters are least divergent. The crosses between the genotypes belonging to distantly located clusters are likely to produce good transgressive segregants and genotypes with better mean values can be selected among the genotypes to suit the breeding programme. Our results are in collaboration with many such studies conducted in pearl millet. In a study by Lakshmana *et al.* (2010) 105 accessions of pearl millet (26 from India and 79 from African countries) were characterized. These accessions were grouped in 22 clusters, indicating the presence of large amount of diversity among the genotypes.

Qualitative characters: Most of the qualitative characters revealed considerable variation except for traits like spike exertion, node pubescence, internode pigmentation, leaf sheath pubescence, anther color, glume pigmentation and plant growth habit (Figure 4). Majority of the lines had complete spike exertion except HSGR-44. Node pubescence was absent and internode pigmentation was green in color in all the inbreds studied. Leaf sheath pubescence were absent in all inbreds. In majority of lines anther color was yellow (except HSGR-17) and glume pigmentation were absent (except HSGR-09). Most of the inbred studied exhibited erect type of growth habit.

Thirteen inbreds (27%) were grouped under presence and remaining thirty six inbreds were grouped under absence (73%) for anthocyanin coloration of first leaf sheath. Forty (82%) inbreds showed absence of spike tip sterility and in remaining nine (18%) inbreds spike tip sterility was present. Eleven inbreds (22%) had green, other eleven (22%) had brown and twenty seven (55%) inbreds had purple plant node pigmentation. Thirty eight (78%) inbreds had absence of spike bristle and for remaining eleven (22%) inbreds spike bristle was present. Eight inbreds (73%) found to have brown and rest three inbreds (27%) had purple bristle color out of eleven inbreds having bristles. Twenty nine inbreds (59%) had cylindrical, eighteen (37%) with conical spike shape, one had candle and rest one had lanceolate spike shape. Two inbreds had loose type of spike, twelve inbreds (24%) had semi-compact, twenty seven (55%) had compact and eight inbreds (16%) were having very compact spike density. Majority of inbreds had grey colored seeds (43%) followed by deep grey (27%) and yellow (22%) and only four inbreds had cream seed color. Globular seed shape (67%) was the most dominant, followed by obovate (33%) seed shape.

The present investigation was conducted to carry out the comprehensive characterization as well as comparative evaluation of pearl millet genotypes based on DUS for application in research, product development, seed production and intellectual property rights (IPRs). On the basis of results, it can be concluded that morphological DUS descriptors can be effectively used for identification, documentation and grouping of varieties along with their use in registration and unambiguous identification in the field. The morphological descriptors would provide a great help to the plant breeders for selecting desirable genotypes easily through visual assessment and further the highly contrasting inbred lines for a particular trait can be utilized to develop mapping populations in order to map QTLs. Although DUS testing has been done precisely to provide diversity assessment at morphological level but there remains a further scope to validate the findings through diversity analysis at molecular level as molecular markers based genetic diversity analyses helps for more effective assessment of genetic relationship among the genotypes.

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Table 1. List of 49 pearl millet stay green inbred lines used for experiment

S. No.	Name of inbred line	Pedigree	S. No.	Name of inbred line	Pedigree
1.	HSGR-01	SPF 2 98-2	26.	HSGR-26	DMRC - 09 / 11-81 -2
2.	HSGR-02	H 90/4-5 X 77/ 29-2	27.	HSGR-27	High Fe JBT / 12 -122
3.	HSGR-03	HTP 92/5	28.	HSGR-28	TPRT / 12- 119
4.	HSGR-04	(ICMB 92333 X EEBC CI-I)-5-B-B	29.	HSGR-29	TCPTA / 12 – 128
5.	HSGR-05	K-560-2X(J 834-7 X 700544-7-2-1)	30.	HSGR-30	110041
6.	HSGR-06	HTP 3/14	31.	HSGR-31	HPT - 2 - 12 -7
7.	HSGR-07	VCF 6862/ 98-1	32.	HSGR-32	99 HS - 22
8.	HSGR-08	AC-04 /6	33.	HSGR-33	2305
9.	HSGR-09	1210/1	34.	HSGR-34	MIR 97041
10.	HSGR-10	H 94/61-2	35.	HSGR-35	G 73 - 107 - 05 K -1
11.	HSGR-11	JBV 3 S1- 44-3-B-4-B	36.	HSGR-36	AC-04 /13
12.	HSGR-12	HTP - 07-26	37.	HSGR-37	99 HS - 23
13.	HSGR-13	HTP 07-44	38.	HSGR-38	99HS -145
14.	HSGR-14	96 AC – 99	39.	HSGR-39	98 Raj 4
15.	HSGR-15	HMP - 0810 (ICMA 01222 X ICMP 451)	40.	HSGR-40	99 ABL - 5
16.	HSGR-16	SPF 2 98-2	41.	HSGR-41	{ICMB 91777 X (91777B X HHVBC)} - 6 -B
17.	HSGR-17	VCF 4 1864	42.	HSGR-42	HBL - 34
18.	HSGR-18	HTP 92 / 110	43.	HSGR-43	1660(M.T.)
19.	HSGR-19	SGP - 10 - 110	44.	HSGR-44	HF IT - 1 -129
20.	HSGR-20	HTP -10 -137	45.	H 77/833-2-202	
21.	HSGR-21	PT - 1-10 -1038	46.	H 77/833 -2	
22.	HSGR-22	PT-1- 10 - 1043	47.	H77/29 -2	
23.	HSGR-23	TCF 3-10-3-2	48.	HBL-11	
24.	HSGR-24	TCF 3-10-28-5	49.	ICMR 01004	
25.	HSGR-25	PT -1-10 - 1099			

Table 2. Grand mean and range for quantitative characters in pearl millet inbreds

S. No.	Characters	Mean ±S.E(m)	Range
1	Days to 50% flowering (days)	46.02± 0.79	40.33-58.33
2	Leaf sheath length (cm)	14.79±0.39	12.67-17.20
3	leaf blade length (cm)	50.88 ± 0.69	33.53-72.13
4	Leaf blade width (cm)	2.68± 0.29	1.14-4.53
5	Number of nodes/ plant	7.51± 0.31	5.20-10.93
6	Spike length (cm)	19.48 ± 0.46	13.60-24.60
7	Spike girth at maximum point (cm)	2.31 ± 0.06	1.22-3.81
8	Number of productive tillers/ plant	4.29 ± 0.20	1.60-9.57
9	Plant height (cm)	135.35 ± 2.41	115.60-160.87
10	1000 grain weight (g)	8.72 ± 0.17	4.35-14.80
11	Dry fodder yield/plant(g)	160.61 ± 2.18	57.74-284.37
12	Green fodder yield/ plant (g)	341.09± 4.03	192.34-517.53
13	Grain yield/ plant(g)	32.24 ± 1.25	21.20-48.47

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Table 3. Cluster means analysis of forty nine inbreds in pearl millet

Traits/ Cluster	Days to 50% flowering (days)	Leaf sheath length (cm)	Leaf blade length (cm)	Leaf blade width (cm)	No. of nodes/ plant	Spike length (cm)	Spike girth at maximum point (cm)	Number of productive tillers/ plant	Plant height (cm)	1000 grain weight (g)	Dry fodder yield/ plant(g)	Green fodder yield/ plant(g)	Grain yield/ plant (g)
Cluster 1	46.97	15.57	45.08	2.29	7.52	18.28	2.11	3.57	129.24	7.56	166.66	295.60	25.18
Cluster 2	42.13	13.56	42.46	1.94	6.61	17.04	1.68	2.73	120.03	7.01	161.00	248.68	25.18
Cluster 3	45.56	14.09	53.05	3.05	7.00	19.30	2.72	4.09	133.65	8.60	153.90	392.63	31.88
Cluster 4	41.66	14.53	60.11	3.13	7.69	19.74	2.57	3.35	129.79	7.39	103.95	249.31	26.57
Cluster 5	50.62	14.85	56.36	2.87	9.18	22.38	2.40	5.95	151.14	11.38	192.31	433.35	44.72
Cluster 6	46.95	15.54	47.85	2.65	6.85	19.74	2.16	5.68	144.51	10.11	178.30	385.01	39.28
Mean	46.95	14.79	50.88	2.68	7.51	19.48	2.31	4.29	135.35	8.72	160.61	341.09	32.24

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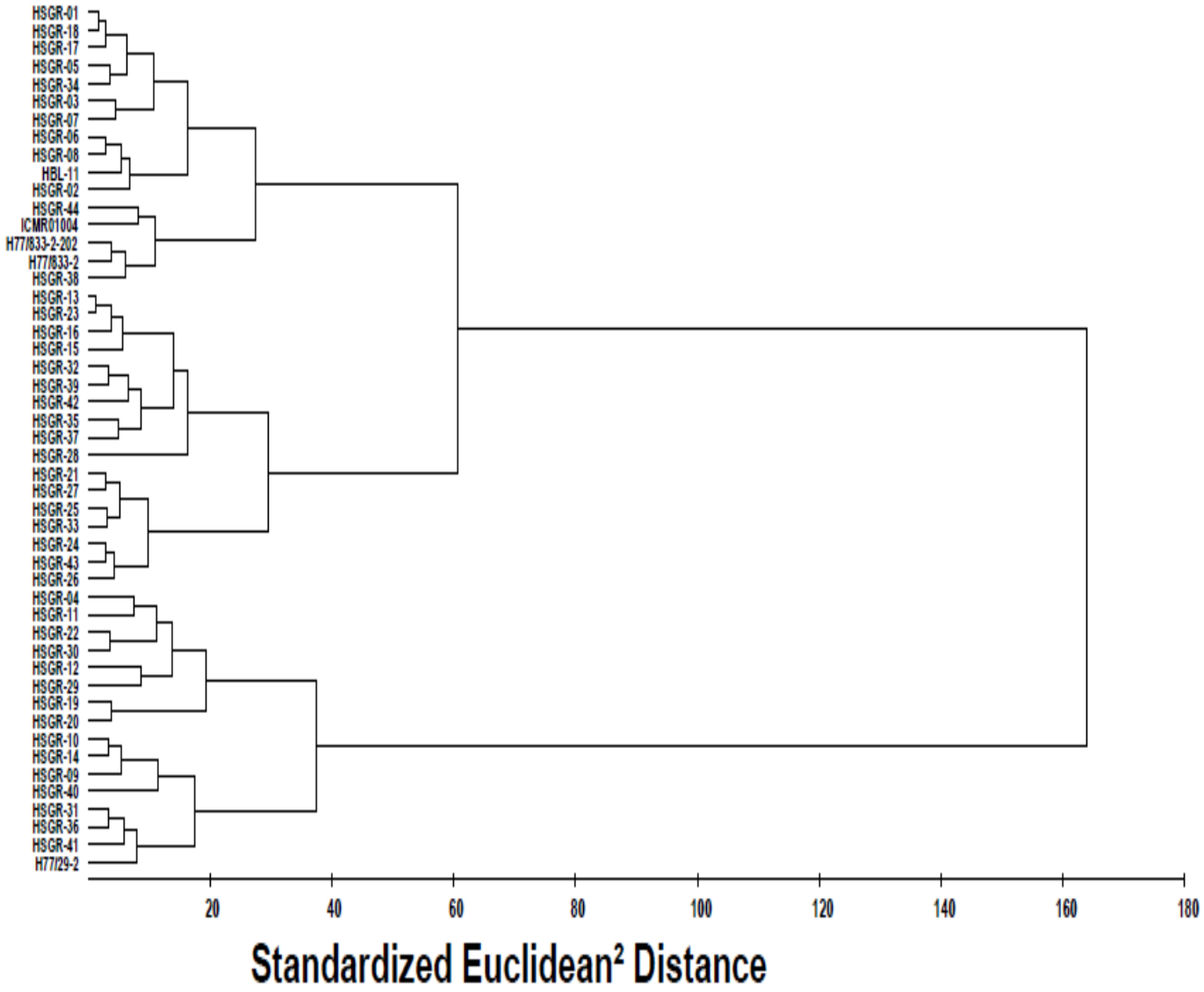

Fig. 1. Dendrogram showing the clustering pattern of forty nine inbreds of pearl millet

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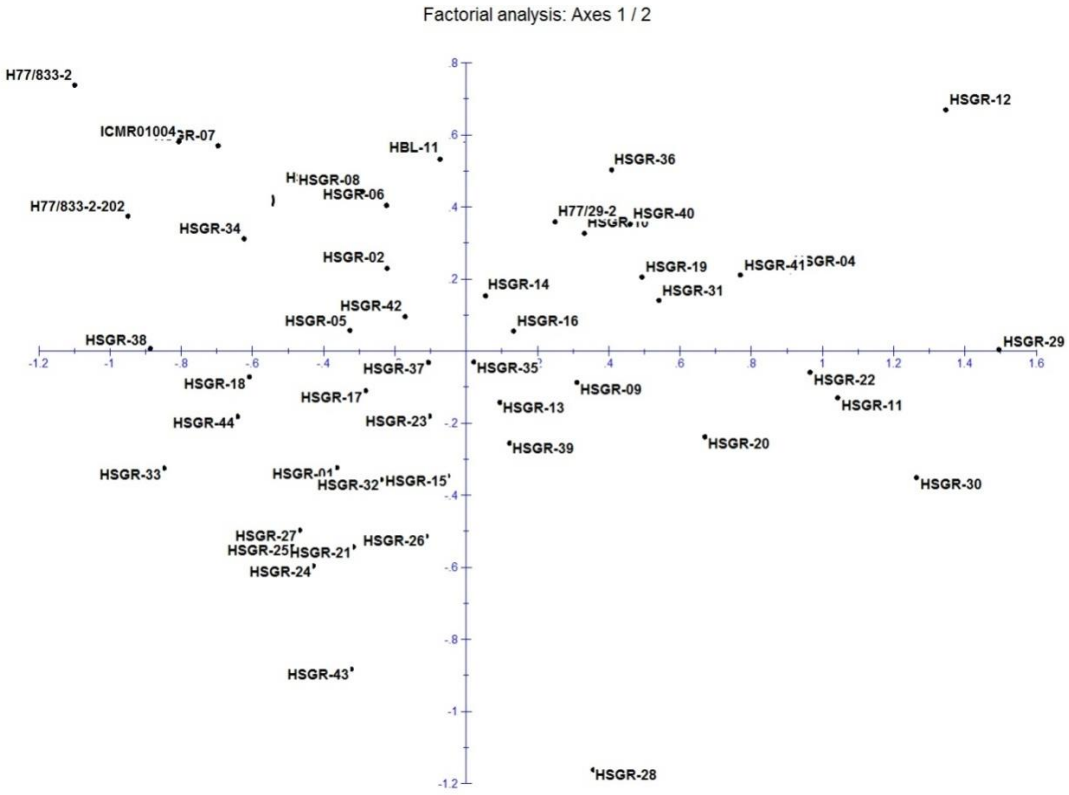

Fig. 2. Principal Coordinate Analysis (PCoA) among 49 inbreds using 13 quantitative traits

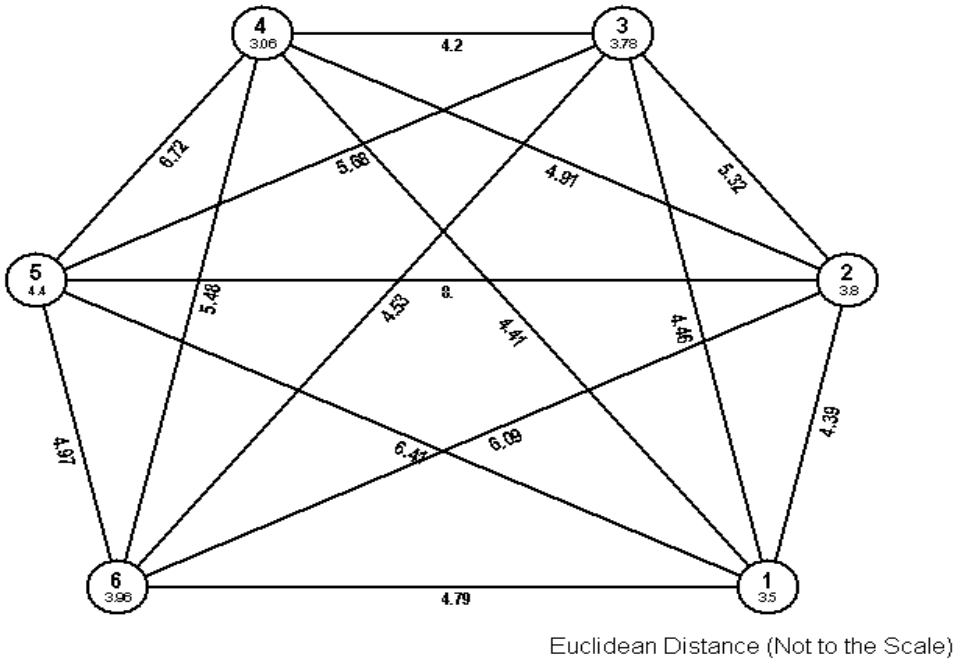

Fig. 3. Intra- and inter- cluster distance for the 6 groups of forty nine inbreds of pearl millet

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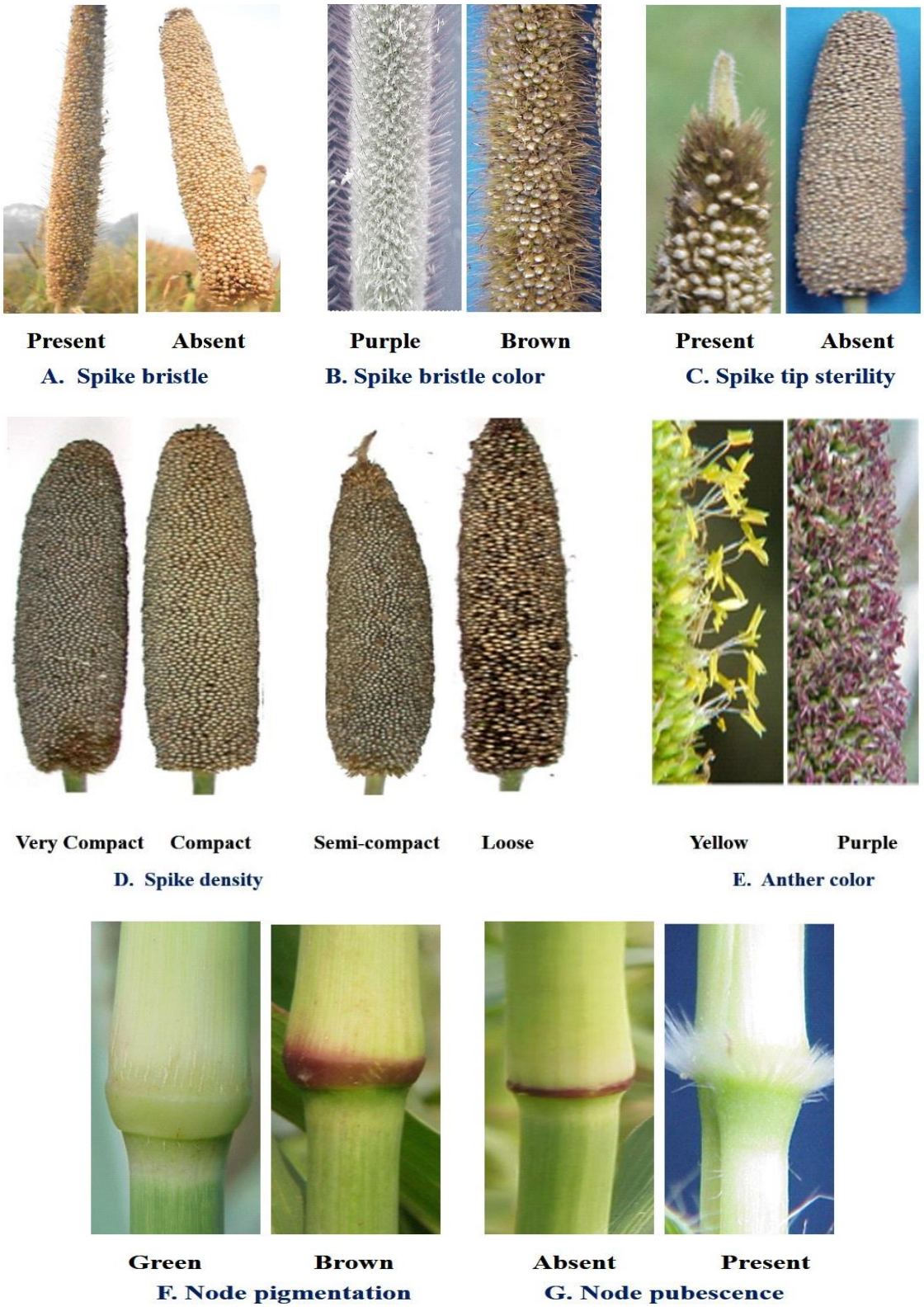

Fig. 4. Spike (A-E) and node (F-G) characterization of pearl millet inbreds