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Original Research Paper

Exploring genetic diversity of Dahlia (*Dahlia variabilis* Desf.) germplasm using multivariate statistics

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ABSTRACT

Dahlia (*Dahlia variabilis*) is a tuberous-rooted flower crop, exhibiting rich diversity in flower color and inflorescence form. The study was conducted to quantify diversity in 24 dahlia genotypes based on agronomic traits. The dahlia accessions were grouped based on their similarity for phenotypic resemblance following hierarchal clustering and principal component analysis (PCA). The hierarchical cluster analysis grouped the dahlia accessions into three distinct clusters *viz.*, C1, C2 and C3 comprising 8, 3 and 13 genotypes, respectively. The 24 dahlia genotypes were found scattered across the whole variation observed by PC1 and PC2 (explaining nearly 55.2% of the cumulative total variation). The two-dimensional PCA analysis revealed that the most appropriate traits for grouping the dahlia accessions were plant height, flower weight, stalk length, vase life and number of flowers per plant. The study signifies the importance of germplasm collection, characterization and utilization of dahlia to popularize its commercial cultivation among the flower growers.

Keywords : Characterization, dahlia, diversity index, germplasm, hierarchal clustering, principal component analysis

INTRODUCTION

Dahlia (*Dahlia variabilis* Desf.) is a tuberous-rooted herbaceous ornamental plant, cultivated for cut flowers and potted flowering plant (De Hertogh, 1996). The genus *Dahlia* comprises 35 species native to Mexico and parts of Central and South America (Slade, 2018). Recent documentation stated 35 wild species of dahlia are endemic to Mexico (Carrasco-Ortiz *et al*., 2019). However, the American Dahlia Society (ADS) has mentioned 42 species (ADS, 2020). It is believed that only four of the species namely, *D. coccinea* Cav., *D. pinnata* Cav., *D. merckii* Lehm. and *D. imperialis* Roezl have constituted the genetic basis for the evolution and development of modern-day dahlia cultivars (Gatt *et al.,* 2000; Hansen and Hjerting, 2008).

Dahlia flowers are appreciated worldwide for their long-lasting majestic blooms exhibiting diversity in flower color, inflorescence form and size, ranging from miniatures (<2.5 cm), to giant (>40 cm). Dahlia is commercially cultivated in Mexico, Japan, France, South Africa, Italy, Germany and the United States with a significant area (400 ha) of bulb production in Netherlands (Priyanka *et al.*, 2017). In India, the commercial cultivation of dahlia is limited to the plains of North-West and Central regions for the domestic flower market. Societies such as American Dahlia Society (ADS), USA; National Dahlia Society (NDS), UK; Dahlia Society of Australia and National Dahlia Society of New Zealand have been collecting and maintaining dahlia germplasm for breeding purpose (Behr and Debener, 2004).

The presence of genetic variability is a pre-requisite for a breeder to evolve varieties exhibiting novel characteristics. The source of variation includes local landraces, exotic germplasm collections, hybrids, improved varieties and mutants evolved as a result of spontaneous or induced mutations. The aesthetic quality traits representing novel flower colour, inflorescence form, uniformity and profuse flowering, longer flower duration, stem sturdiness with long vase life and floral scent hold considerable significance and forms a major breeding objective for the dahlia breeders (Marina, 2015; Dalda Şekercil and Gülşen, 2016).

The characterization of germplasm based on flower color, inflorescence form and other agronomically important traits is essential to explore the potential parentage for breeding Dahlia. The present study was carried out to analyze variability in Dahlia germplasm for inflorescence characteristics and field-based

agronomic traits through multivariate data analysis statistical tools (Rencher, 2002). The results of multivariate statistical analysis of germplasm generates a relevant and precise information on morphological diversity in different traits of dahlia germplasm, that will help in the selection of desirable accessions for utilization in future breeding programs targeting a specific trait(s). The studies on these aspects is of substantial significance and will also help in promoting the cultivation of dahlia crop with small and marginal farmers, especially in developing countries, for diversification in their traditional cropping systems and for generating additional income.

MATERIALS AND METHODS

The experiment was conducted at the Research Farm, Department of Floriculture and Landscaping, Punjab Agricultural University (PAU), Ludhiana. Twenty-four dahlia genotypes comprising of six cultivars *viz.*, Earl Haig, First lady, Hammett 96, Ice berg, Sam Hopkins and Sister Nivedita and 18 genotypes collected from secondary sources especially from local nurseries and designated as accessions numbers (D1, D2…). Six rooted cuttings each of 24 genotypes were planted at 45 cm x 30 cm in randomized block design (RBD) replicated thrice. The recommended cultural practices pertaining to the crop were followed at different growth stages. Pinching was done when plants attain 4-5 pairs of leaves. Observations were recorded for 11 quantitative traits *viz*., plant height (cm), number of primary branches, number of flowers per plant, flower diameter (cm), flower weight (g), stalk length (cm), days to first bud emergence, days to full bloom, duration of flowering (days), days to flower withering and vase life (days).

Dahlia accessions were classified for different inflorescence forms and flower size as a qualitative descriptor and were assigned codes following the guidelines proposed by ADS and NDS. Data were statistically analyzed using IBM SPSS v22 statistical software (IBM Corp, 2013) and agricolae statistical package developed by De Mendiburu and Yaseen (2020).

RESULTS AND DISCUSSION

Vegetative and floral characteristics

Dahlia genotypes revealed significant (pd”0.05) differences for vegetative and floral characteristics (Table 1). Quantifying the variability present in the traits of agronomic importance help breeders and growers to make a selection of specific genotype(s) pertaining to their objective of breeding (Carrasco-Ortiz, 2019). The plant height ranged from 39.27 cm to 103.30 cm, genotype D8 recorded the maximum number of primary branches (4.67) and minimum in D2 (1.00). The variation in number of primary branches can be attributed due to pinching of terminal growing shoots (for breaking the apical dominance) to promote sprouting of lateral buds. The uniform plant architecture in dahlia is maintained by retaining 3-5 well spaced primary branches. The greater number of branches leads to higher leaf biomass that enhances the flower and tuber yield by regulating the source-sink relationship (Manjula *et al.,* 2017). However, the results revealed that the pinching of apical shoots delayed flowering by 5-10 days and the duration of the plants were longer as compared to non-pinched plants as also reported by Miller and Filios (2011) in Dahlia. The number of flowers per plant varied from 1.67 (D7) to 12.50 (D5), flower diameter ranged 12.45 cm (Earl Haig) to 20.23 cm (Ice Berg), flower stalk length ranged 15.10 cm - 58.40 cm indicating wide diversity in dahlia genotypes for varying length of flower stalks. Dahlia variety ‘Earl Haig’ and accession D9 took maximum (92.00) number of days to bud emergence. Days to full bloom varied from 74.70 to 112.40 days. The genotypes D 15 (57.33 days) and D1 (60.90 days) were found early flowering and at par with each other. The duration for days to flower withering ranged 93.60 days (D1) to 136.33 days (D17) to and vase life from 3.20 days (D9) to 6.30 days (D20). Dahlia genotypes exhibiting both early and late emergence of buds are advantageous to get the blooms earlier and later growing season, respectively (Priyanka *et al*., 2017). Selection of accessions initiating buds deviating from the peak season are preferred by the breeders to evolve varieties that can capture the market early (Hamrick, 2003). Surprisingly, 1/3rd of the accessions revealed variation for duration of flowering, which can be attributed due to recurrent blooming habit of dahlia, that continues to flower profusely when old and withered flowers are periodically removed from the plant (Romer and Nelson, 2008).

The information on the traits such as stalk length and vase life are important for breeding dahlia to evolve novel cut flower types with longer stem length and enhanced life of cut flower (Armitage, 1993). Among the genotypes tested, 14 accessions recorded longer vase life (>4 days), signifying the identification and

Table 1 : Performance of dahlia genotypes for growth and flowering parameters

Genotype	Plant height (cm)	No. of primary branches	No. of flower per plant	Flower diameter (cm)	Single flower weight (g)	Stalk length (cm)	Days to first bud emergence	Days to full bloom	Duration of flowering (days)	Days to withering	Vase life (days)
Earl Haig	49.20	1.50	4.50	12.45	22.70	34.55	92.00	111.00	39.66	131.66	3.20
First Lady	60.23	4.33	7.33	15.67	30.73	30.00	79.66	107.50	52.34	132.00	5.50
Hammett 96	63.97	2.00	4.67	20.00	54.67	29.63	80.66	112.40	47.00	127.66	3.80
Ice Berg	90.67	2.00	9.33	20.23	65.20	32.93	70.00	95.40	52.00	122.00	5.20
Sister Nivedita	74.93	1.33	6.33	17.57	36.23	18.17	62.00	85.00	65.33	127.33	5.50
Sam Hopkins	58.23	1.33	2.67	14.23	37.23	48.87	72.66	85.90	39.34	112.00	4.20
D 1	103.30	1.70	8.80	16.60	46.70	15.30	60.90	74.70	32.70	93.60	3.70
D 2	100.70	1.00	5.90	18.20	52.50	16.80	62.30	77.00	44.80	107.10	5.20
D 3	87.90	3.00	7.40	20.50	53.30	15.10	65.30	80.60	42.30	107.70	6.20
D 4	68.53	1.33	5.67	13.77	44.60	43.30	84.66	101.20	41.67	126.33	4.80
D 5	60.80	3.30	12.50	19.90	55.90	23.60	62.70	76.30	34.20	96.90	5.70
D 6	59.33	1.33	4.33	16.17	41.97	22.17	78.33	94.00	37.67	116.00	3.50
D 7	58.83	1.67	1.67	15.93	44.47	55.67	81.00	98.20	39.00	120.00	5.20
D 8	42.57	4.67	8.00	16.27	23.43	14.57	65.33	104.12	55.00	120.33	3.50
D 9	49.20	1.50	4.50	12.45	22.70	34.55	92.00	111.00	39.66	131.66	3.20
D 13	72.36	1.67	8.33	12.50	33.80	21.06	65.33	77.78	42.44	107.78	5.83
D 14	52.63	1.67	3.00	14.90	57.30	17.97	80.00	100.40	42.33	122.33	3.50
D 15	39.27	3.00	6.67	13.23	26.23	24.57	57.33	80.20	64.67	122.00	3.50
D 17	68.97	2.67	3.33	15.67	70.30	59.30	91.00	112.40	45.33	136.33	4.80
D 18	98.77	1.33	3.67	19.73	38.07	47.33	67.00	92.40	56.33	123.33	5.20
D 19	53.70	3.00	8.00	14.03	40.73	39.47	75.00	92.40	53.66	128.66	3.50
D 20	75.37	4.00	3.67	20.00	46.30	33.53	80.66	110.40	55.34	136.00	6.30
D 22	64.70	1.67	2.67	13.93	24.27	58.40	83.33	97.00	45.33	128.66	3.80
D 24	60.60	2.33	3.33	13.23	31.37	48.07	70.00	94.10	60.00	130.00	4.20
LSD ($p<0.05$)	12.59	2.02	4.01	1.54	8.13	11.94	8.41	9.33	7.35	7.35	0.71

selection of desirable parents for the hybridization programme. The stalk length is another important post-harvest trait relevant for breeding cut flower dahlia and the variation in stalk length might due to the genetic make of the genotypes. The length of stem is considered as an oligogenic trait, governed by few genes, whereas the flower color is considered as a polygenic trait.

Dahlia genotypes revealed variation with respect to inflorescence type and days to flowering duration. The inflorescence of dahlia was classified based on the guidelines proposed by ADS and NDS classification system (Table 2) with their respective size codes. The inflorescence forms were classified as Formal Decorative (FDi), Informal Decorative (ID), Fimbriated (Fim.), Single (S), Stellar (ST), Collarette (CO), Peony (PE) and Double Orchid (DO). The DO form of inflorescence recorded highest diameter of flower (19.90 cm) followed by Fim (16.73 cm) and ID (16.62 cm) types, while CO group recorded smallest flower diameter (13.77 cm). The variation in flower diameter is important from breeding perspective as the blooms with larger and more number of petals tend to offset selfing with greater probability of out-crossing. The increase in petallage number (doubleness of flowers) and length act as a barrier for the transfer of pollen from disc florets to petal stigmas, thus increasing the chances of out-crossing (Vinanyananda, 1993; Phetpradap, 1992). The count of ray and disc florets significantly affects the weight of flower which in turn is influenced by the variation in temperature. Generally, a night temperature of 16-21^{o}C resulted in increase in the number of ray and disc florets (Moser and Hess, 1968). The inflorescence forms of dahlia were accorded *per cent* values computed against the total number of accessions evaluated. Around 41.6% accessions exhibited FDi form of inflorescence followed by 20.8% accessions revealing ID. Around 4.16% of the total accessions revealed each of the ST, PE, CO and DO forms of inflorescence. The ST form of inflorescence was not designated under NDS guidelines.

The ADS recognizes 18 types of inflorescence forms of dahlia and 9 classes for flower size ranging from 2 to over 10 inches in diameter. The type of inflorescence also determines the breeding system in dahlia that determines its potential to set seeds. Most of the dahlia are believed to be cross compatible (where bees and ants facilitate the cross pollination) with fewer (<25%) cultivars being self-compatible (Behr and Debener, 2004). The current breeding programmmes aim for evolving dahlia suitable for cut flower with novel

Table 2 : Classification of 24 dahlia accessions based on inflorescence form and size

Genotype (Nos.)	Accessions/ genotype	Classification based on ADS guidelines			Classification based on NDS guidelines		
		Inflorescence description					
		Average diameter (cm)	Form	Abbr. codes	Form	Abbr. codes	% of total accessions
10	Earl Haig, First Lady, Hammett 96, Sister Nivedita, D1, D2, D7, D8, D9, D14	16.18	Formal Decorative	FDi	Decorative, small	SD	41.6
5	Ice berg, Sam Hopkins, D4, D5, D6	16.62	Informal Decorative	ID	Decorative, medium	MD	20.8
3	D3, D15, D18	16.73	Fimbriated	FIM.	Fimbriated	FIM.	12.5
1	D17	15.93	Stellar	ST	-	-	4.16
1	D19	15.67	Peony	PE	Paeony	Pae.	4.16
1	D13	13.77	Collarette	CO	Collarette	Col.	4.16
1	D20	19.90	Double orchid	DO	Decorative, medium	MD	4.16
2	D22, D24	14.85	Single	S	Single	Sin.	8.33

ADS- American Dahlia Society, USA; NDS-National Dahlia Society, UK

flower color and inflorescence form accompanying variation in foliage architecture and foliage color as well (Hammett, 2009).

Correlation coefficients and principal component analysis

The simple correlation coefficients computed for the quantitative descriptors revealed highly significant ($p<0.05$) positive correlations (Table 3) for the growth and yield parameters. Plant height recorded significant positive correlation with flower diameter and single flower weight. Number of flowers per plant registered significant and negative association with stalk length, days to bud emergence, days to full bloom and days to withering. Flower diameter had significant positive correlation with single flower weight and stalk length had significant and positive association with days to first bud emergence, days to full bloom and days to withering. Days to first bud emergence revealed a negative correlation with number of flowers per plant followed by plant height, flower diameter and number of primary branches, but was found significantly positively correlated with stalk length. Days to full bloom was found significantly positively correlated with days to first bud emergence followed by stalk length. Negative correlation of days to full bloom was observed with number of flowers per plant followed by plant height.

Duration of flowering was found positively correlated with number of primary branches followed by days to full bloom and flower diameter. However, it was found negatively correlated with days to first bud emergence, individual flower weight and plant height. Days to flower withering was found significantly positively correlated with days to full bloom, days to first bud emergence and duration of flowering. It was found negatively correlated with number of flowers per plant, plant height, flower diameter and individual flower weight. The vase life showed significantly positive correlation with plant height, flower diameter, single flower weight, number of flowers per plant and duration of flowering, but was found negatively correlated with days to first bud emergence and days to full bloom followed by days to flower withering. Similar results were also obtained by Lal *et al.* (1982) in rose flower diameter, and Sirohi and Behera (2000) chrysanthemum vase life. Correlation analysis revealed that selection of characters that are positively correlated can lead to concomitant increase in either of the traits and is a potentially feasible tool to selection of dahlia genotypes for planned hybridization programmes.

To identify the most influential traits governing the greater proportion of variation, multivariate statistical analysis was performed to delimit the large number of variables using PCA analysis. Four PCs explained 82.4% of total variation with lowest component variance (0.104) recorded in PC4. The PC-1 accounted for 37.1% of total variation that included flower retention and longevity traits (number of flowers, plant height, flower diameter and vase life). The traits pertaining to flowering such as duration of flowering, number of flowers, and number of primary branches contributed to PC-2 revealing 18.1% of total variability. The PC-3 described 16.8% of the total variation exhibited, largely contributed by vegetative and post-harvest characters like number of primary branches, Duration of flowering and vase Life. Around 10.4% of the total variation was addressed by PC-4 primarily dominated by plant height, stalk length and duration of flowering (Table 4).

The PCA analysis revealed that the most appropriate traits for grouping dahlia genotypes were plant height, flower weight, stalk length, vase life and number of flowers (contributing for ten genotypes), days to first bud appearance was found shared by 5 genotypes and traits comprising duration of flowering and number of primary branches were governed by five genotypes. It can be inferred that the PC1 and PC2 were best PCs suggesting these as a good reference for aiding the selection of genotypes for future breeding programs.

Hierarchical cluster analysis

Hierarchical cluster analysis aided in grouping of 24 dahlia genotypes into three distinct clusters *viz*., C1, C2 and C3. Cluster C1 comprised eight dahlia genotypes namely D1, D2, D3, D5, D13, D18, Sister Nivedita and Ice berg, whereas, three genotypes (D8, D15 and D19) formed cluster C2. Cluster C3 was observed largest of the three clusters consisting of 13 genotypes (D4, D6, D7, D9, D14, D17, D20, D22, D24, First Lady, Earl

Table 3 : Pearson's correlation between 11 morphological traits of Dahlia accessions

Parameter	Plant height (cm)	No. of primary branches	No. of flower/ plant	Flower diameter (cm)	Single flower weight (g)	Stalk length (cm)	Days to first bud emergence	Days to full bloom	Duration of flowering (days)	Days to flower wither ing	Vase life (days)
Plant height (cm)	1										
No. of primary branches	-0.308	1									
No. of flower/plant	0.169	0.376	1								
Flower diameter (cm)	0.589**	0.201	0.281	1							
Single flower weight (g)	0.474*	-0.061	0.142	0.608**	1						
Stalk length (cm)	-0.129	-0.173	-0.596**	-0.268	-0.033	1					
Days to first bud emergence	-0.356	-0.118	-0.563**	-0.336	-0.026	0.552**	1				
Days to full bloom	-0.403	0.207	-0.486*	-0.125	-0.071	0.408*	0.845**	1			
Duration of flowering (days)	-0.100	0.334	-0.039	0.064	-0.249	0.015	-0.304	0.081	1		
Days to flower withering	-0.399	0.157	-0.538**	-0.252	-0.219	0.509*	0.663**	0.825**	0.513*	1	
Vase life (days)	0.533**	0.192	0.235	0.563**	0.388	-0.050	-0.282	-0.258	0.102	-0.173	1

* Significant at p=0.05, ** significant at p=0.01

Table 4 : The representation of variability from first four PCs from PCA analysis of eleven quantitative traits in dahlia accessions

Principal components (PCs)	Plant height (cm)	No. of primary branches	No. of flowers/ plant	Flower diameter (cm)	Weight of flower (g)	Stalk length (cm)	Days to 1st bud emergence	Days to full bloom	Duration of flowering (days)	Days to with-ering	Vase life (days)	Variabi-lity (%)	Cumu-lative (%)
PC1	0.317	0.022	0.344	0.300	0.205	-0.306	-0.404	-0.395	-0.054	-0.406	0.260	0.371	0.371
PC2	-0.390	0.180	0.254	-0.373	-0.497	-0.304	-0.275	-0.213	0.152	-0.129	-0.333	0.181	0.552
PC3	-0.080	0.580	0.149	0.288	0.013	-0.058	-0.094	0.226	0.541	0.341	0.280	0.168	0.720
PC4	0.279	-0.379	-0.337	-0.099	-0.271	0.256	-0.320	-0.296	0.537	0.134	0.142	0.104	0.824

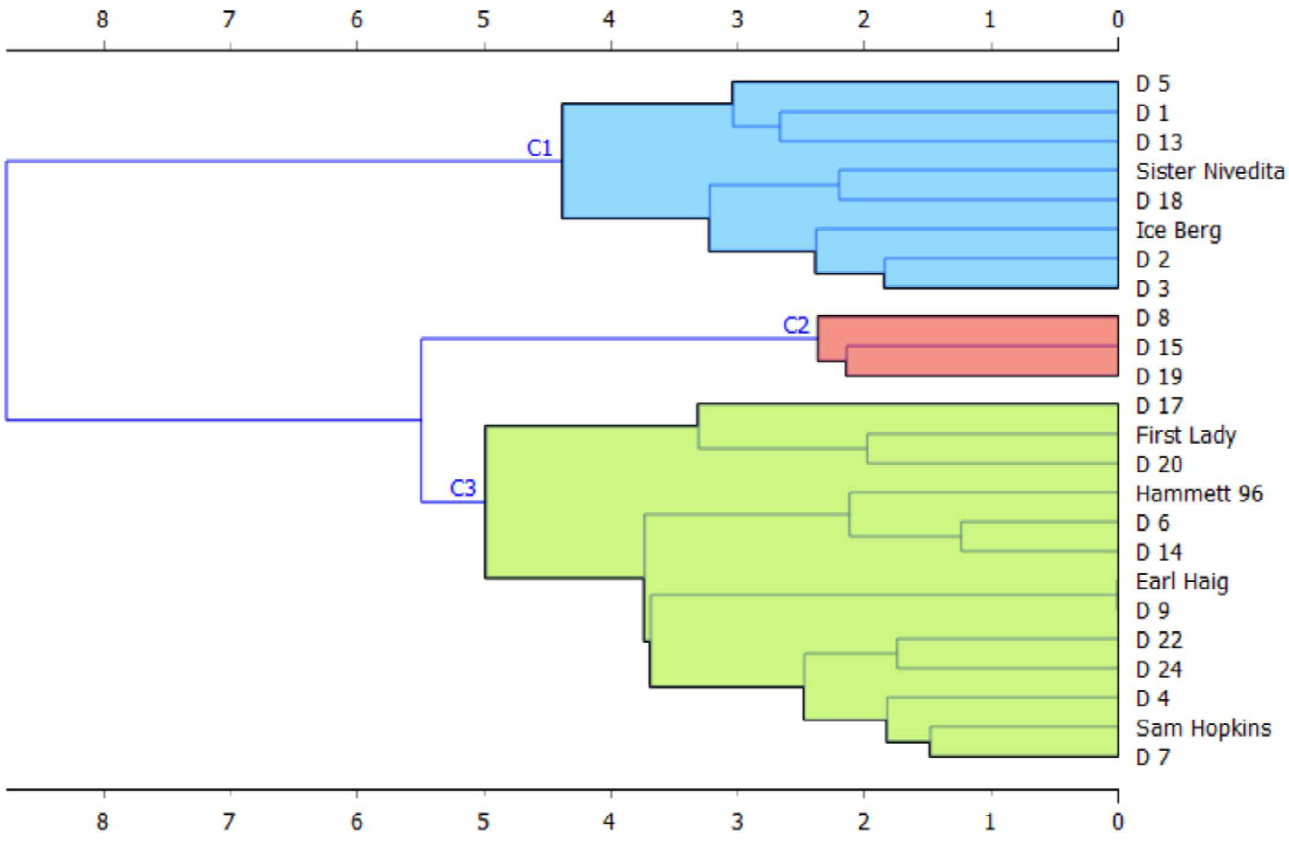

Fig. 1 : Dendrogram presenting genetic relationship among 24 Dahlia accessions

Haig, Hammett 96 and Sam Hopkins) (Fig. 1). The 3 clusters were also found in near perfect accordance with PCs, PC1 and PC2 (contributing 55.2% of the total variability) represented by 2-D plots based on performance of accessions for various quantitative traits (Fig. 2). The genotypes D4, D7, D14, D20, D22 and Hammett 96 from cluster C3 revealed no variation for both PC1 and PC2, whereas, genotypes D22 from C3 cluster reported minimal variation for both the PC components. The genotype D17 (termed as outlier) reported negative correlation for both the PCs. The genotypes D8 and D15 from cluster C2 reported variation of higher magnitude for PC2 and contributed minimal variation for PC1. The genotypes D2, D3, D18 and Ice berg from the cluster C1 reported positive variance for PC1.

The large variation was recorded by flower weight, number of flowers, days to 1st bud emergence, flower diameter and plant height as mentioned by the relative length of the vectors in the biplot diagram (Fig. 3). The biplot also revealed the trait relationship and positive association among the flower weight, plant height, flower diameter, vase life and days to withering, days to first bud emergence and stalk length as indicated by the acute angle. The biplot representing clustering of dahlia accession for different traits revealed interesting results that were found adhering to cluster analysis (Fig. 3). The genotypes from cluster C3 revealed similarity for days to flower withering, days to first bud appearance, and stalk length. The accessions from cluster C2 exhibited similarity for number of primary branches and duration of flowering. The accessions from C1 cluster were observed similarity for traits such as plant height and number of flowers. The accessions from the cluster C3 such as D4, First Lady, Sam Hopkins and D14 are similar and closer to the center and may not have environmental influence. The genotypes in the cluster C2 exhibited the highest yield and associated traits such as number of flowers per plant, flower weight, plant height and vase life. These genotypes can be selected as parent for the future breeding programme to develop high yielding cut flower varieties with better vase life.

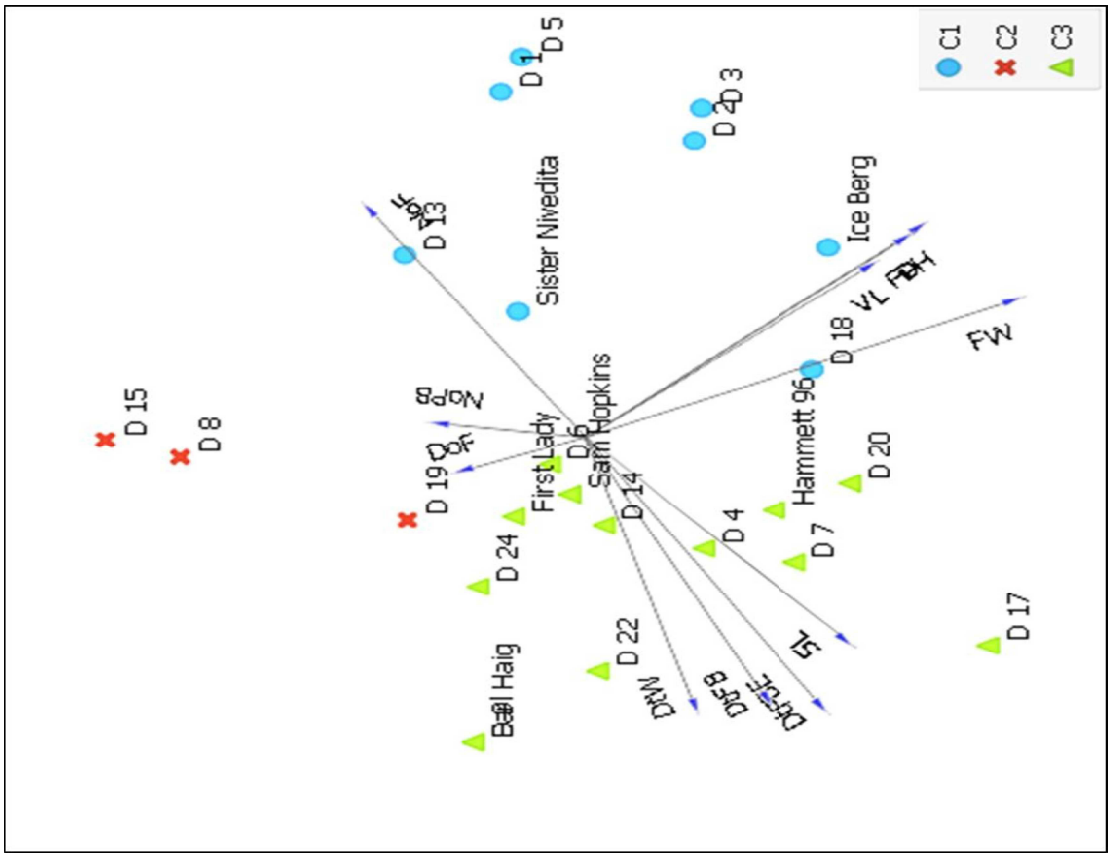

Fig. 3 : Biplot presenting the variable projection of the 24 Dahlia accessions characterized by mean plant height, number of flowers per plant, duration of flowering, number of primary branches and days to first bud appearance. [PH- plant height; NoPB- number of primary branches; NoF- number of flowers; FD- flower diameter, FW- flower weight; SL- stalk length; DtFBE- days to first bud emergence; DtFB - days to full bloom; DoF- duration of flowering; DtFW- days to flower withering; VL- vase life]

Fig. 2 : Scatter diagram for 1st and 2nd PCs for 11 morphological traits in 24 Dahlia accessions

CONCLUSION

The study undertaken describes the morphological assessment of dahlia germplasm based on multivariate statistical methods. The perusal of data presented for agronomic traits will help to characterize the germplasm collection and also aid in selection of suitable lines catering to specific breeding objectives. Our study was able to discriminate different dahlia accessions based on the variability in quantitative and qualitative traits, which would help breeders and growers to make a selection of specific genotype pertaining to their breeding objective.

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