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Full Length Article

Determination of Heterotic Groups among Sunflower Accessions through Morphological Traits and Total Seed Storage Proteins

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Abstract

Ninety seven sunflower accessions/lines were analyzed for developing heterotic groups through agromorphological and total seed storage proteins through SDS-PAGE. Data for nine morphological parameters were recorded from the field and were used for constructing the dendrogram using UPGMA for an insight of the level of genetic distance among the studied material and its genetic potential for seed yield and its related traits. Correlation coefficient estimates showed that seed yield per plant had a positive association with 100 seed weight, head diameter, number of leaves per plant, leaf area, plant height and stem curvature while a negative association with flowering time. Likewise, seed storage proteins were also used to assess the genetic diversity among the sunflower material as proteins are a reliable source of studying the genetic distance among the plants because they are least affected by the environmental factors. Results showed the appearance of 25 protein bands out of which 16 were polymorphic and rest monomorphic. Data of polymorphic bands were used to draw dendrogram to distribute the material into various groups. Both the dendrograms were then compared, and it was found that male and female lines were divided into different clusters with few exceptions. After comparing the dendrogram and morphological data for seed yield and its related attributes six female and six male lines were recommended so that unnecessary crosses could be avoided thus saving the time and cost of developing and finding a suitable commercially viable sunflower hybrid.

Keywords: Heterotic groups; SDS-PAGE; Sunflower; Diversity; Correlation

Introduction

Sunflower (*Helianthus annuus* L.) is the world's 4th important oilseed crop (Masvodza *et al*., 2014), According to botanical classification sunflower belongs to family Asteracea, tribe Heliantheae, sub-tribe Helianthae that contains 20 genera (Deshmukh *et al*., 2016). The total world production of sunflower oil during 2015–16 was 15854000 tons (FAS, USDA, 2016) that accounts for about 14% of the total oil production. Beside this sunflower also contributed to oil cake of about 7% worldwide (Masvodza *et al*., 2014). In Pakistan this crop was grown on 214000 acres with production of 92000 tons seed from which about 35000 tons of vegetable oil was extracted during 2015–16 (Pakistan Economic Survey, 2015–16).

Owing to its importance agricultural scientists have been trying since early 1900's to exploit its full potential as this crop has wider adaptability range for soils and climates therefore can be successfully cultivated in tropics and sub-tropics all around the year (Sunil *et al*., 2013). Presence of genetic diversity is a pre-qualification for having a variety of better traits in any crop improvement program (El-Zeadani *et al.*, 2014; Shinwari *et al*., 2014; Jan *et al.*, 2017a, b). To have a successful breeding program we have to find the diversity among the genotypes under investigation (Sultan *et al*., 2013). In sunflower, line/genotype characterization is mainly performed through agro-morphological traits as these are easy to record and require less technical hands and labor, but on the other hand, these markers are less in number and also highly affected by the environment (Lochner, 2011).

For characterization of genetic diversity and determination of differentiation among plants biochemical markers like proteins are another powerful tool (Jan *et al.*, 2016a; Jan *et al.*, 2017a). SDS PAGE is a biochemical method commonly used for assessment of genetic composition of a crop germplasm. This procedure separates the seed storage proteins of crop under research (Khan *et al*., 2013), that assists in determining the evolutionary and taxonomic relationships of the crop and its wild and domesticated relatives (Shinwari *et al*., 2014).

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In the present investigation 97 sunflower accessions were divided into different heterotic groups through agro-morphological markers and the genetic distance among the sunflower lines under investigation were also counter determined by the SDS-PAGE as well. The objectives of this research was to (i) determine the level of genetic dissimilarity among the sunflower lines through agro-morphological and protein markers,(ii) assigning the genotypes into different heterotic groups based on the protein markers and agro-morphological characterization and (iii) suggesting various cross combinations based on the genetic distance and yield performance for finding the superior hybrid combination.

Materials and Methods

Plant Material

For agro-morphological description ninety eight sunflower genotypes were used in the present study, and field experiment was performed during Spring-2015 at National Agricultural Research Centre (NARC), Islamabad, Pakistan. Two rows of each entry were sown with R × R distance was 75 cm and P × P distance was maintained at 25 cm.

Morphological Measurements

All necessary agronomic practices were performed to ensure a healthy crop stand. Data was recorded for days to start of flowering, days to flower completion, plant height, stem curvature, leaf area, number of leaves per plant, 100 seed weight, head diameter and seed yield per plant. Data were averaged to compute the means of all the traits so that genetic distance among the sunflower accessions under investigation could be computed.

Total Seed Storage Proteins Analysis through SDS-PAGE

Seeds of 97 accessions of sunflower were used for estimation of genetic diversity among them through SDS-PAGE analysis. Buffer for extraction of proteins was prepared from 0.5 M Tris HCL pH 8.0, 0.2%SDS, 5M Urea and 1% β-mercaptoethanol. For the purpose of visualization of movement of proteins in the gels, Bromophenol Blue (BPB) is also added to the extraction buffer. Electrode buffer solution was composed of (0.025 M Tris, 0.125% SDS and 0.129 M glycine) Tris 30 g, glycine 144 g, SDS 12.5 g and d. H2O 900 mL.

For the extraction of sunflower seed proteins, 1–2 seeds of each line was de-hulled and ground to fine powder using pestle and mortar. A 0.2 g of seed flour was then mixed with 400 mL of protein extraction buffer in 1.5 mL of eppendorf tube. Samples were then homogenated by centrifugation at 12000 rpm for 10 min. at room temperature and stored at -4^{o}C till further process.

SDS-PAGE of total seed proteins was performed in vertical slabs according to the protocol described by Jan *et al.* (2016b). Polyacrylamide (12.5%) separating gels was formulated having 30% and 0.135% by weight acrylamide and N-N-methylene-acrylamide resp. used in 3M Tris HCL having pH 8.8 with 0.4%. However, 30% acrylamide and 0.8% N-N-methylene bis-acrylamide in 0.493 M Tris HCl containing 0.4% SDS was used for stacking gel preparation. Ammonium per sulfate (10%) and 17 µL TEMED was added for polymerization of gel. Gel electrophoresis was done at 100 V till the BPB marker reached at the bottom of the gel. In order to check the molecular weights of the dissociated polypeptides, pre-standard protein ladder ranging from 10–180 kDa (Lot: 00345 035, Thermo scientific) was used.

As most of the proteins are colorless, staining was done to visualize the separated polypeptides. For this purpose the staining solution was prepared that contains 0.25% (w/v) Commasie Brilliant Blue (CBB), 10% (v/v) acetic acid, 40% (v/v) methanol diluted in water. Staining of gels was done for 3–4 h so that CBB dye should bind with the proteins. Gels were then transferred into the de-staining solution to wash away any extra CBB from the gels. The de-staining solution contained 5% acetic acid (v/v), 20% methanol (v/v) and distilled water in 5:20:75 ratios. Gels washed in this solution till the background color disappeared and protein bands are clearly visible.

Data Analysis

Data recorded were subjected to two statistical analyses namely, Cluster analysis and Principle Component analysis to visualize the level of diversity among the studied material, so that recommendations could be made for development of hybrid combinations on the basis of genetic divergence among the sunflower genotypes. All the data analyses were carried out through statistical package PAST version 3.14.

For proteins data, on the basis of presence (1) and absence (0) of proteins bands, the bands were scored and a binary data matrix was generated and similarity index was calculated for all possible pairs of bands. This data was further used for calculating the Euclidean distance matrix which was further employed for cluster analysis making a dendrogram tree through UPGMA following Sneath and Sokal (1973). Dendrogram was constructed using statistical package PAST version 3.14.

Results

Morphological Characterization

Results of the mean, range, C.V and heritability estimates are presented in Table 1. All the nine agronomic parameters used in the present experiment showed a great deal of genetic variation among the studied material as it is revealed by the C.V and their mean values.

Table 1: Mean Performance of Sunflower Accessions for nine agro-morphological traits during 2015

Accession	DFI	DFC	P.H	S.C	H.D	L/P	L/A	100 s.w	S.Y/P
CMS-HAP-12	84	93	223.58	33.23	17.6	32.2	240.89	6.578	58.79
CMS-HAP-56	80	89	200	23.67	18.3	28	255.43	5.589	65.35
CMS-HAP-101	85	97	236	21	17.6	33.8	247.84	4.5	44.446
CMS-HAP-54	81	92	209.8	24.8	18.4	32.6	257.77	5.34	64.14
CMS-HAP-103	79	91	207.2	32.1	19	32.8	161	5.01	37.42
CMS-HAP-24	75	89	200.5	30	12.75	45.75	253.12	5.82	42.142
CMS-HAP-110	80	91	201	33.6	16.4	34.6	284.77	5.126	44.478
CMS-HAP-112	80	90	198	35.17	19.6	33.6	217.55	5.227	63.97
CMS-HAP-111	82	91	141	34	17.8	25.8	237	5.68	56.06
CMS-HAP-114	75	84	215	21	17.2	36.2	228.6	5.49	32.466
CMS-HAP-115	75	84	123	33.02	18.2	23.6	203.11	5.197	33.697
CMS-HAP-03	85	94	229	33	17	32	244.75	4.5	42.646
CMS-HAP-99	83	93	212.5	21.25	23.75	33	259.67	6.663	57.886
CMS-HAP-118	79	87	173	35	15	27.8	214.33	5.59	33.19
CMS-HAP-125	81	94	200	34.3	22.4	33	237.33	8.108	26.267
CMS-HAP-116	80	90	194	35.09	18.89	32	215.33	5.236	41.76
CMS-HAP-121	81	88	177	34	18.6	26.6	279.89	7.128	47.367
CMS-HAP-117	81	87	191	27	15.6	27.6	251.56	6.813	38.54
CMS-HAP-122	84	91	170	34	17.8	30.8	238.11	4.764	40.73
CMS-HAP-120	77	86	212	26.1	13.4	30	229.39	4.6	38.796
CMS-HAP-123	78	84	200.5	33.55	16.8	33.6	265.43	7.93	38.12
CMS-HAP-102	71	76	173.7	24.9	16.3	27.5	251.789	4.947	40.67
CMS-HAP-119	81	87	161	24	15.4	30.4	218.56	4.984	42.75
HAP-12	75	89	206.4	34	15.4	33.8	155.55	5.052	33.067
HAP-56	80	92	207	33.47	17.2	32	184.89	4.731	40.96
HAP-101	87	97	190	26	15.8	27.8	280.13	5.53	42.558
HAP-54	81	93	218.5	20	16	34	266.78	5.899	48.433333
HAP-103	79	92	221	33.56	16.8	34.6	163.28	5.907	50.283333
HAP-24	77	93	202	25	13.2	40.2	224.3	4.96	42.124
HAP-110	80	92	214	33	16.8	35.2	208	5.834	43.4
HAP-112	81	88	195	34.6	19	34.6	151.89	4.648	33.38
HAP-125	83	98	191	35	17	28.4	200.44	3.63	43.97
HAP-102	78	86	182	33.7	13.4	23.2	263.41	6.25	41.808
HAP-114	83	91	165	20.67	13.8	25	174	4.48	33.526
HAP-116	81	89	170	35	15.4	27.6	197.22	5.3	40.34
HAP-99	81	91	146	22.9	16	24	158.11	4.84	27.316667
HAP-123	75	96	218	25	21.4	36.8	192	3.85	42.12
HAP-03	78	83	243.6	22.5	17.2	35.4	228.51	5.19	40.792
HAP-08	74	86	189.76	33.3	13.9	33.3	239.61	5.25	38.49
RHP-68	81	95	167	21.3	15.6	27.6	184.89	4.4	29.976667
RHP-53	96	115	196	26	16.6	33	198.33	4.58	37.14
RHP-72	91	99	162	21.2	11	27.6	161.67	3	44.546667
RHP-46	85	94	183	23.1	9	26.8	259	2.91	29.743
RHP-76	81	93	190	19.23	10.2	26.8	170.44	3.28	27.2
RHP-41	91	99	199	21.1	11	33	154.67	3.9	29.153333
RHP-81	92	108	164	20.6	12.4	30.6	212.33	3.1	26.206667
RHP-69	91	100	179	22.3	10.6	34.4	151.33	3.46	27.81
RHP-38	83	92	172	21	11	26.4	157.34	4.51	37.676667
RHP-73	81	93	166	20.7	9.4	29.4	148.89	2.83	31.146667
RHP-74DNDN	81	93	193	22	12.8	32.6	167.78	4.3	30.61
RHP-74DN85	97	118	188	22.2	11.6	30.6	184	4.28	29.82
RHP-74DN90	91	102	178	21.8	9.2	29.6	219.11	3.92	28.357
RHP-74DN95	85	100	194	20.3	10.2	28.2	216.22	4.15	29.416
RHP-74DN98	83	99	200	21.5	11	31.8	152.33	5.45	27.923333
RHP-74DN100	85	97	183	22.34	9.4	26.4	153.44	3.98	30.955
RHP-74DN105	91	111	192	22.6	11.6	28	179.22	4.23	29.96
RHP-74DN107	90	100	185	23.7	10.8	29	152.89	3.745	30.25
RHP-74DN108	91	98	173	22	10	26.8	167.22	4.49	28.74
RHP-74DN110	93	118	205	23	10.2	33.4	164	4.15	30.72
RHP-74DN112	96	115	206	21.6	8.8	31.6	171.78	3.9	28.4
RHP-74DN115	83	99	185	21.2	9.8	27	172.44	3.92	30.25
RHP-74DN120	85	101	197	26	10.6	29.4	158.22	3.96	29.7
RHP-74DN125	81	97	158	21.67	9.6	25	152.89	3.81	30.0833
RHP-74DN128	91	101	193	21	10	36.8	160.56	3.95	29.5167
RHP-74DN130	90	98	175	21.1	8.6	28	157.11	4.45	28.245
RHP-71	90	98	156	24.5	13	26	160.33	3.42	32.2
SFP-14	75	86	225	24.23	15.2	36.6	251.69	4.1	42.168
SFP-12	79	84	202	34	17.2	33.4	241.72	4.93	37.708
SFP-10	77	84	198	35	13.8	30	238.23	5.38	37.838
SFP-40	81	91	179	22	17.6	31.2	194.77	5.99	30.267
SFP-42	74	83	251	18	18.2	34.6	253.9	5.5	37.902
SFP-38	78	96	241	20	16.8	33.2	222.1	5.91	37.928
SFP-18	79	90	236	22	16.6	31.4	256.7	5.3	41.014
SFP-36	79	93	196.6	23	16.8	35.8	133.22	7.98	45.6
TSFP-31	71	78	146	22.2	15.8	26.4	254.47	6.39	37.86
SFP-37	79	90	184	23	17	31.2	127.22	5.134	42.35
SFP-23	78	86	247	21.34	16.4	29.2	227.53	6.54	42.486
SFP-09	78	95	197.4	31.25	14.25	37	244.71	5.1	40.16
SFP-41	77	98	234	34.75	15.5	34.75	250.01	5.18	38.008
SFP-19	78	98	221.6	30.75	14.5	30.75	277.58	5.39	39.602
SFP-22	77	86	163.4	33.68	15.1	36.7	178.356	5.793	39.48
SFP-25	73	78	131.1	27.3	15	30	220	5.4	39.432
SFP-43	74	81	160	30	17	31.8	178.2	5.12	43.29
SFP-33	85	95	235	25.6	16.8	37	260.67	5.9	41.64
SFP-46	75	83	138	34	17.4	25.2	150.22	4.624	44.63
SFP-08	81	89	128	21.46	14.2	26.6	145.33	6.564	40.637
SFP-07	74	84	188	32.8	17.2	31.8	274.62	5	42.51
SFP-16	74	84	235	22.14	18.4	29.4	233.6	6	40.138
SFP-26	77	87	153.21	24	18.1	31.2	163.39	5	40.798
SFP-13	89	98	241	23.6	17.6	30	227	4.39	41.346
SFP-35	73	78	240	29	19	36.2	221.92	4.98	42.238
SFP-87	75	85	140	25	15.8	31.2	162	5.469	40.85
SFP-32	75	93	210	31.2	16.2	39.8	227.98	6.26	41.128
SFP-05	76	84	131	34.9	15.6	24.4	152.33	4.956	37.73
SFP-06	74	84	167	22	10.6	26.4	233.71	4.1	38.806
SFP-20	74	80	232.5	31	22.4	38.4	248.69	6.186	42.144
SFP-86	80	93	152	29	16.2	28	246.61	6.18	38.144
Min	71	76	123	18	8.6	23.2	127.22	2.83	26.20667
Max	97	118	251	35.17	23.75	45.75	284.77	8.108	65.35
Mean	81.18	92.28	191.24	26.65	15.09	31.08	207.92	5.04	38.62
C.V %	7.30	8.91	15.42	20.56	22.42	13.25	20.47	21.21	21.34

Days to flower initiation (DFI); Days to flower completion (DFC); Plant height (PH), Stem curvature (SC); Head diameter (HD); Number of leaves per plant (L/P); Average leaf area (L/A); 100 seed weight (100 s.w); Seed yield per plant (S.Y/P)

SFP-31 and CMS-HAP-102 takes the least number of days for start of flowering i.e., 71 days while RHP-7485 takes 97 days to first flowering thus late in initiation of flowering. CMS-HAP-102 days was found to be the earliest in completion of flowering as it takes only 76 days for all the plants of the entry to flower. RHP-7485 and RHP-74110 took most number of days i.e., 118 for all the plants in the entry to flowering. CMS-HAP-115 was found to be the shortest stature plant and had a height of 123 cm while SFP-42 was the tallest plant under investigation that recorded the height of 251 cm (Table 1).

Head diameter has a positive association with the seed yield per plant as larger head accommodate more number of seeds and possibly larger seed size. But too large head size increases the risk of lodging. Head size in the studied sunflower accessions ranged from 8.6 (RHP-74130) to 23.75 (CMS-HAP-99). A 100 seed weight ranged from 2.83 to 8.108 g while maximum seed yield recorded was 65.35 g for CMS-HAP-56 and minimum seed yield observed was 26.20 g exhibited by RHP-81 (Table 1).

Seed yield is considered to be the trait of prime importance, therefore, the genotypes that exhibit higher level of seed yield potential could be considered for utilization in further hybrid breeding programs. Six female genotypes those showed the highest seed yield/plant and six male lines with highest seed yield/plant are recommended for further utilization in hybrid breeding program to study the efficacy of heterotic groups in sunflower. Six elite lines were recorded that could be useful for further development of hybrid combinations in L x T Fashion (Table 5).

Table 2: Correlation coefficient estimates of nine agro-morphological traits

Characters	DFI	DFC	P.H	S.C	H. D	N/P	L/A	100 s.w	S.Y / P
DFI		0.86974	-0.02208	-0.34518	-0.49164	-0.16372	-0.30808	-0.44614	-0.33493
DFC			0.098576	-0.32166	-0.47625	0.001183	-0.29629	-0.42638	-0.35206
P.H				-0.04854	0.26413	0.58614	0.38825	0.15819	0.20270
S.C					0.44309	0.13124	0.24896	0.34144	0.32786
H. D						0.27606	0.39905	0.6014	0.59909
L/P							0.19544	0.21071	0.18489
L/A								0.404	0.43844
100 s.w									0.41933

Days to flower initiation (DFI); Days to flower completion (DFC); Plant height (PH), Stem curvature (SC); Head diameter (HD); Number of leaves per plant (L/P); Average leaf area (L/A); 100 seed weight (100 s.w); Seed yield per plant (S.Y/P)

Table 3: Grouping of 97 sunflower accessions based on morphological analysis

Cluster	Sub-Group	Accessions	No. of Genotypes	Percent %
I	*A*	*HAP-1104, RHP-68, SFP-40, SFP-22, SFP-43, RHP-72, RHP-71, RHP-38, RHP-73, RHP-74125, RHP-76, RHP-74DN, RHP-74115, RHP-41, RHP-7498, RHP-74120, RHP-74128, RHP-69, RHP-74130, RHP-74100, RHP-74107, RHP-74108*	22	22.68
	B	*RHP-53, RHP-7485, RHP-74105, RHP-74110, RHP-74112*	5	0.062
	C	*SFP-36, SFP-37, CMS-HAP-103, HAP-12, HAP-1102, HAP-103*	6	0.062
	D	*HAP-99, SFP-26, SFP-87, SFP-46, SFP-05, SFP-08*	6	0.062
II	*A*	*HAP-54, SFP-19, CMS-HAP-56, CMS-HAP-54, CMS-HAP-99, CMS-HAP-24, CMS-HAP-123, CMS-HAP-125, CMS-HAP-117, HAP-08, SFP-12, SFP-10, SFP-09, CMS-HAP-1104, CMS-HAP-120, HAP-24, SFP-32*	17	17.52
	B	*CMS-HAP-110, HAP-101, CMS-HAP-121, SFP-07, HAP-102, RHP-46*	6	0.062
	C	*HAP-03, SFP-37, SFP-16, SFP-35, SFP-38, SFP-13, CMS-HAP-101, SFP-18, SFP-33, CMS-HAP-03, SFP-41, SFP-14, SFP-20, CMS-HAP-12, SFP-42*	15	15.46
	D	*CMS-HAP-1102, CMS-HAP-116, HAP-25, HAP-56, HAP-247, HAP-110*	6	0.062
	E	*CMS-HAP-118, HAP-245, CMS-HAP-122, CMS-HAP-119, SFP-06, RHP-81, RHP-7490, RHP-7495*	8	0.082
	F	*CMS-HAP-111, SFP-31, SFP-86, CMS-HAP-115, SFP-25*	5	0.052

Correlation Analysis

Linear correlation coefficient was estimated among the studied traits and results are presented in Table. 2. Results revealed that Days to start of flowering showed a negative correlation with all the traits studied except Days takes to full flowering. Likewise, highest correlation with seed yield per plant was exhibited by the head diameter (0.5990). Similarly, average leaf area (0.4384) and 100 seed weight (0.4933) also showed a higher degree of association with seed yield per plant in positive direction.

Hierarchical Cluster Analysis on Agro-morphological Characterization Basis

Euclidean distance matrix estimated from the means of nine morphological traits studied were used to construct the dendrogram to assess the genetic similarity among the sunflower accessions so that these can be divide into various heterotic groups on the basis of genetic distance among them. Dendrogram depicted an overall genetic distance of 90%, which implied that the genotypes had a 10% similarity among them on the basis of agronomic traits. The dendrogram constructed divided the genotypes into two main groups (Fig. 2). Cluster 1 had 39 lines while cluster 2 had 57 lines. All the restorer lines were clustered into cluster 1 and most of the female lines were grouped into cluster 2 with few exceptions wherein, RHP-46, RHP-81, RHP-7490 and RHP-7495 were found in cluster 2 and CMS-HA 103 was found in cluster 1. Cluster 1 showed 4 sub-groups and cluster 2 can be further sub-divided into 6 sub-groups. Cluster 1-A has 22 genotypes, similarly cluster 1-B, 1-C and 1-D contains 5, 6 and 6 genotypes, respectively. Likewise Cluster 2 was also sub-divided into 6 sub-groups wherein cluster 2-A had 17 no. of accessions grouped together while cluster 2-B, 2-C, 2-D, 2-E and 2-F had 6, 15, 6, 8 and 5 sunflower accessions respectively. Genotypes in one group were more closely related to one another than the genotypes in the adjacent groups. Similarly, the groups of accessions were more distantly related the sunflower accessions. Table 3 showed the distribution of sunflower lines into various clusters/groups as indicated by the tree diagram (Fig. 2).

Cluster Analysis on Protein Banding Basis

SDS-PAGE analysis yielded 25 bands of varying weights and of these 25 bands 16 had shown polymorphism while rests are monomorphic. Only polymorphic polypeptides bands were used for constructing the tree diagram from cluster analysis.

Using UPGMA, hierarchical clustering was done and a dendrogram was constructed (Fig. 3) that divided the sunflower accessions depending on the difference between the protein banding patterns. Protein dendrogram showed a genetic distance of less than 3% indicating that the accessions had more than 97% similarity index as revealed by their protein bands.

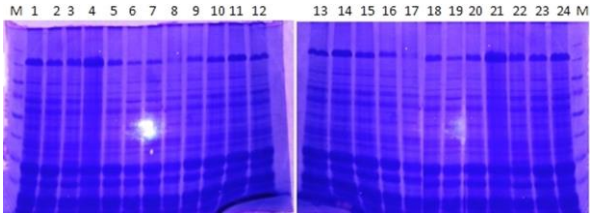

Fig. 1: Protein banding pattern of some sunflower genotypes as revealed by SDS-PAGE analysis

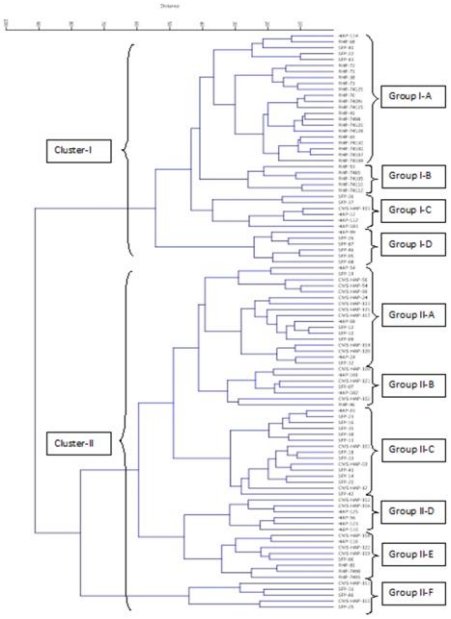

Fig. 2: Dendrogram of Ninety seven sunflower accessions used for morphological analysis

The tree diagram showed that accessions were divided into two main groups wherein restorers and female lines divided into different groups. Three sub-groups could be seen in the each main group. The Group-I contained 43 accessions and all the male lines i.e., restorers were clustered together in this Group-I. This cluster was sub-divided into three classes, wherein group I-A had 15 genotypes, group II-B contained 17 genotypes while the group I-C had 11 genotypes clustered together (Table 4).

The other main cluster contains total of 53 genotypes. Along-with other sunflower accessions all CMS lines under study were clustered in this Group II. This group was also sub-divided into three smaller classes based on the magnitude of difference in the seed protein banding patterns of sunflower. The group II-A had 10 genotypes, while the second group II-B contained 19 sunflower lines. The third group designated as group II-C showed 24 sunflower genotypes clustered together closely (Table 4).

Discussion

Morphological characterization is an important breeding

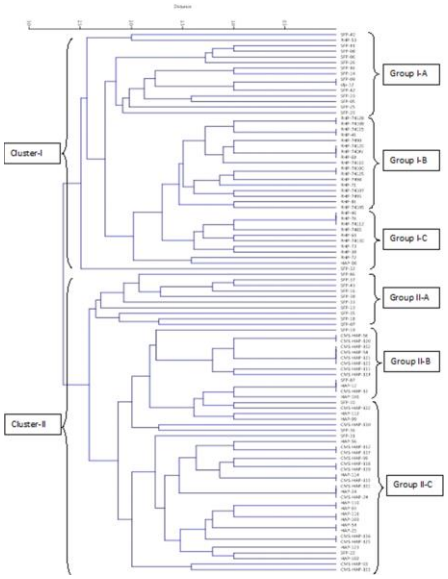

Fig. 3: Dendrogram of Ninety seven sunflower accessions used for SDS-PAGE analysis

practice as it identified the character-specific genetically distant and morphological similar and better sunflower genotypes (Masvodza *et al.,* 2014). Results of agronomic parameters showed a considerable genetic variability among the studied material (Table 1). Since the genetic distance is a measure of genetic variability (Cheres *et al.,* 2000; Nasreen *et al.,* 2011), a good possibility of exploitation of heterosis among the sunflower material under investigation existed. Moreover, performance of a cross also depends on the genetics potential of its parents (Sujatha and Nandini, 2002). Genotypes showing a good performance for the seed yield per plant could be efficiently utilized in future sunflower breeding programs. Correlation estimates give information of the relationship in the various plant traits. As previously reported (Masvodza *et al*., 2014), the results of the present study indicated that seed yield per plant had a positive association with most of plant traits except days to flower initiation and completion (Table 2). Thus the efforts should be made to combine the alleles of seed yield and early maturity so that high performing early maturing sunflower hybrids could be developed.

Cluster analysis results revealed that genotypes exhibit considerable variation on the basis of either male or female lines, although some exceptions also existed (Fig. 2). Hierarchical clustering indicates the level of genetic variability among the lines and this genetic information can be efficiently used in practical plant breeding (Sultana *et al.,* 2006). It is generally believed that a superior could be developed by combining the two genetically distant parents (Nasreen *et al.,* 2011). Thus cluster analysis provided useful information about the genetic variability of the lines under study (Nasreen *et al.,* 2011). The inbred lines that

Table 4: Grouping of 97 sunflower accessions based on SDS-PAGE analysis

Group	Sub-group	Accessions	No. of genotypes	Percent%
I	*A*	*SFP-40, RHP-53, SFP-41, SFP-08, SFP-06, SFP-26, SFP-46, SFP-14, SFP-09, SFP-12, SFP-42, SFP-37, SFP-05, HelL-215, SFP-20*	15	15.26
	B	*RHP-74128, RHP-74108, RHP-74115, RHP-41, RHP-7490, RHP-74120, RHP-74DN, RHP-68, RHP-74110, RHP-74100, RHP-74125, RHP-7498, RHP-71, RHP-74107, RHP-7495, RHP-81, RHP-74105*	17	17.71
	C	*RHP-46, RHP-76, RHP-74112, RHP-7485, RHP-69, RHP-74130, RHP-73, RHP-38, HA-92, HAP-08, SFP-32*	11	11.45
II	*A*	*SFP-86, SFP-37, SFP-43, SFP-16, SFP-38, SFP-33, SFP-14, SFP-35, SFP-18, SFP-07*	10	10.42
	B	*SFP-19, CMS-HAP-56, CMS-HAP-120, CMS-HAP-102, CMS-HAP-54, CMS-HAP-121,CMS-HAP-123, CMS-HAP-111, CMS-HAP-1104, SFP-87, HAP-12, CMS-HAP-12, HAP-101, SFP-10*	19	19.79
	C	*SFP-31, HAP-56, CMS-HAP-1102, CMS-HAP-117, CMS-HAP-99, CMS-HAP-118, CMS-HAP-119, HAP-1104, CMS-HAP-115, CMS-HAP-101, HAP-24, HAP-110, HAP-03, HAP-245, HAP-103, HAP-54, HAP-25, CMS-HAP-116, CMS-HAP-125, HAP-247, SFP-22, HAP-102, CMS-HAP-03, CMS-HAP-103*	24	25.0

Table 5: Lines Recommended for further development of hybrid combinations in L x T Fashion

CMS Lines	Restorer Lines
CMS-HAP-12	*RHP-68*
CMS-HAP-56	*RHP-53*
CMS-HAP-54	*RHP-41*
CMS-HAP-1102	*RHP-69*
CMS-HAP-111	*RHP-38*
CMS-HAP-99	*RHP-71*

show a considerable genetic distance, coupled with superior performance for economically important traits like seed yield and its controlling characters, may be used in sunflower hybrid/varietal improvement program (Nasreen *et al.*, 2011).

Relationship within and between taxa can be studied through SDS-PAGE technique as it is a reliable approach for estimating the genetic distance among the members of a plant population (Jan *et al.*, 2016a). However, during the present study a limited intra-specific variation was observed at protein level through SDS-PAGE (Fig. 1). All the genotypes were classified into in two major groups (Fig. 3). A similar type of protein banding pattern has been observed in sunflower by Zilic *et al*. (2010) and found that although almost all genotypes showed similar number of protein bands, but most of them were polymorphic and characteristic of a specific hybrid; hence SDS-PGAE could be efficiently utilized for differentiation studies. According to Awan *et al.* (2017), molecular markers are used for selection of better wheat genotypes.

Conclusion

Both protein and morphological characterization was utilized to identify different heterotic sunflower groups. It is concluded that mating among partners that had shown superior performance for seed yield/plant and were also distant male and female genotypes are more suitable for further mating purposes. The elite lines could be further utilized in an L x T mating design for studying the efficacy of genetic distance and seed yield/plant as the predictor of performance of hybrids in sunflower.

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