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Genetic diversity analysis of local and foreign sunflower germplasm *(Helianthus annuus)* for the national breeding program: Zimbabwe

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Characterisation of germplasm resources is vital for efficient management of breeding programs. Commonly used methods include biochemical, morpho-agronomic, molecular and to some extent cytological characterisation. With an effort to rescuscitate an ailing national sunflower breeding program after a series of major droughts, 16 female (A) lines and 10 male (R) lines were morphologically characterised. Ten morphological variables namely days to 50% flowering, head diameter, leaf length, leaf width, petiole length, nodding, lodging, number of leaves, plant height, stem diameter and uniformity were used for characterisation. Data was collected and analysed using Genstat software. After an ANOVA, principal components analysis (PCA) and hierarchical cluster analysis were used to examine distribution patterns in the germplasm. Significant differences were observed for the variables ($p<0.001$). Hierarchical clustering on morphological data indicated an overall similarity index of 91%, that is, 9% dissimilarity was observed. For PCA, it can be observed that the cytoplasmic male sterile (CMS) lines are more spatially distributed as compared to the R-lines. The genetic base of the collection was observed as narrow and would need more diversification otherwise inter-crosses will not produce much gain.

Key words: Morphological variables, compositae family, principal components analysis, cluster analysis.

INTRODUCTION

Sunflower (*Helianthus annuus* L.) is one of the four most important oilseed crops in the world which include rape oilseed, soyabean and cotton (Yadava et al., 2012). Sunflower accounts for about 14% of the world production of seed oils and about 7% of the oilcake and meal produced from oilseeds. Sunflower is a preferred source of oil for domestic consumption and cooking worldwide (Kholghi et al., 2011). It is generally preferred for its light colour, high level of unsaturated fatty acids, good flavor and high smoke point (Hu et al., 2010).

Sunflowers have been grown since 1900 in Zimbabwe but sunflower research started at Harare in 1975 in response to improved interest in sunflower by local farmers and oil expressers (Oilseed Handbook, 1990). A

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Table 1. List of female lines used in the study consisting of local and foreign lines.

S/No	Type	Lines	Local/foreign	S/N	Type	Lines	Local/foreign
1	A	CMS HA 290	L	9	A	807-73-9-10	L
2	A	CMS HA 89	L	10	A	CMS124 X Oroka	L
3	A	CMS AP822-4-2-2-1	F	11	A	CMS HA 236	L
4	A	CMS AP996-12-1-3-3	F	12	A	807-73-11-10-2-2	L
5	A	H55-2-2	L	13	A	CMS124 X HS 20	L
6	A	H55-9-3-1	L	14	A	CMS124X Issanka	L
7	A	497-74-9-4-2	L	15	A	CMS228	L
8	A	808-73-9-1	L	16	A	79-75-7-2	L
17	R	RP953-29-1	L	18	R	RHA363	F
19	R	RHA279	L	20	R	RHA368	L
21	R	RHA294	L	22	R	RHA309	L
23	R	RHA340	L	24	R	RHA857	L
25	R	RHA344	L	26	R	RHA297	L

R denotes restorer lines; A denotes a cytoplasmic male sterile line.

few hybrid varieties of sunflower are released each year by the national breeding programme in Zimbabwe. Consequently, there are limited varieties and seed on the market making the seed expensive (CBI, 2000). Farmers resort to use of retained seed which is less productive and open pollinated varieties which are generally less uniform (Murata et al., 1997). A lot of sunflower germplasm need to be characterized for morpho-agronomic traits and molecular properties for efficient use in the breeding program (CBI, 2000).

Characterization is important in describing accessions, establish their diagnostic characteristics and identify duplicates; classifying groups of accessions using sound criteria; identifying accessions with desired agronomic traits and select entries for more precise evaluation, developing interrelationships between, or among traits and between geographic groups of cultivars and estimating the extent of variation in the collection (Khan et al., 2014; Kholghi et al., 2011). It aims at identifying trait-specific genetically diverse and agronomically similar or better sunflower germplasm lines.

Estimating genetic diversity and determining the relationships among germplasm collections enhances efficient collection, management and genetic improvement (Khan et al., 2014). Plant breeders can also use genetic similarity information to complement phenotypic information in the development of breeding populations (Poehlman and Sleper, 1998). Multivariate statistical techniques, which simultaneously analyze multiple measurements on each subject under investigation, are widely used in analysis of genetic diversity, whether, morphological, bio-chemical or molecular marker-based. Among the multivariate techniques, cluster analysis, principal component analysis (PCA), principal coordinate analysis (PCoA) and multidimensional scaling (MDS) are most commonly employed and appear particularly useful (Mohammadi and Prasanna, 2003; Grahic et al., 2013). A preliminary morphological characterization is the purpose of this study to explore diversity patterns within a collection of local and a few foreign lines.

MATERIALS AND METHODS

Plant material

Sixteen cytoplasmic male sterile (CMS) lines and ten male restorer (R) lines were used in the study (Table 1). Among the lot are two South African CMS lines and one restorer line meant for evaluation under local conditions. The local lines were obtained from the national breeding programme in Zimbabwe. The local lines were originally acquired from various sources and were retained in the program since the 80's.

The female lines have a male sterility trait which means they cannot produce viable pollen. This trait is useful for hybrid seed production. They are each maintained by a twin maintainer line which is exactly the same as it is genotypically, except on a single or few loci for production of viable pollen. The restorer lines, on the other hand, characteristically can restore pollen fertility in the hybrid progeny if used as male parents in a cross. The two traits, male sterility and restoration are critical in the breeding of sunflower. The restorer lines used in the study are shown in Table 1. Characterization was carried out in the department of Crop Science, University of Zimbabwe.

Experimental design

For morphological characterization the inbred lines were grown in black polythene bags each measuring 33 cm diameter and 34 cm depth. They were planted in a completely randomized design replicated three times, with five pots per plot. Red clay topsoil was dug out and mixed thoroughly and then used to fill up thirty pots. Initially three seeds were planted per pot and then thinned to two

Table 2. Morphological character descriptor list.

Variable	Description
Lodging score (LDG)	Extent of lodging 1 for upright, 3 moderately lodged, 5 highly lodged
Number of leaves (NL)	Total number of true leaves
Nodding (ND)	Angle of nodding of a mature head, 1 good, 3 moderate and 5 bad
Head diameter (HD)	The longest distance passing through the centre of the sunflower head
Petiole length (PL)	Distance from stem to leaf blade (cm)
Leaf width (LW)	Length of widest leaf part (cm)
Leaf length (LL)	Distance from leaf base to leaf apex (cm)
Stem diameter (SD)	Widest point of the stem from the base (cm)
Plant height (PHT)	Distance from the base to the apex of the plant (cm)
Days 50% flower (DAYF)	Number of days when half of the plants have flowered
Uniformity score (U)	1 for highly uniform to 5 for non-uniform

plants per pot at four weeks after emergence. The pots were arranged in rows 60 cm apart and 30 cm in within row.

Compound D fertilizer (N: 7, P205:14, K20:7) was applied as a basal dressing at 8 g/pot. Ammonium nitrate (34.5%N) topdressing was applied as a side dressing at four weeks after emergence. All pots were watered to field capacity every three days. No watering when effective rainfall was carried out. Weed control was carried out hand pulling weeds in all pots as when necessary in order to maintain weed free pots at all times.

Variables

Eleven morphological traits were evaluated as indicated in Table 2. For data collection five plants were randomly selected and scored in each plot to effect analysis of variance. Analysis of Variance (ANOVA) was carried out for all measurements using Genstat Discovery Edition. After an ANOVA all means were captured and used for two multivariate methods namely, principal components and hierarchical cluster analysis to explore diversity patterns.

RESULTS AND DISCUSSION

Significant differences were observed for the eleven morphological variables, $p<0.001$ for leaf length, leaf width, number of leaves, petiole length, stem diameter, and plant height and head diameter. For the rest of the variables, nodding, uniformity, days to flowering and lodging a $p<0.01$ was obtained after a two way ANOVA using genstat software. The means and standard error are presented in Table 3.

Out of the eleven variables, only seven have a direct agronomic importance namely, days to flowering, head diameter, nodding score, lodging score, plant height and uniformity. Days to flowering have an implication on earliness of a variety. Some very early varieties may record around 55 days to flowering, while moderate ones, 65 to 75 days and late varieties 75 days and above. In the study a mean of 67.55 shows a moderate range. The collection has a mix for all ranges and selection is not limited. The imported varieties, RP953-29-1, has 64 days and is closer to CMS HA290, a standard line, which has 66 days. Another standard line CMS HA89 has 87 days. CMS HA290 and CMS HA89 are officially released cultivars on the market. In the collection some very early lines are 807-73-11-10-2-2 with 54 days and 807-73-9-10A with 55 days. The short and medium maturity groups suit the environment in Zimbabwe because seasons are short.

Head diameter has an implication on yield as they accommodate many and possibly large seeds. However, too large heads may increase lodging or may have hollow centres. Further analysis is necessary during selection. None of the new lines have outstanding head diameter most likely because they are inbred lines. Lodging is also affected by plant height and stem diameter and indirectly related to nodding, besides head diameter. The new female lines both show good scores of 1 for lodging, whereas the male line has a score of 4.

The angle at which the head nods at maturity will make the seeds either easy access for bird predation or can be overbearing on the stem resulting in weak stems. During selection the nodding score is taken into account. Of the three foreign lines, two namely RP953-29-1 and AP822-4-2-2-1, have a score of 4 and only one, AP996-12-1-3-3, has a good score of 2. The standard lines CMS HA290 and CMS HA89, do not show any good scores for nodding with a 4 and a 5 respectively. Too tall plants with thin stems are more likely to lodge. An average lodging score of 1.93 shows a good standability of the collection. Extreme scores of fives and fours, have however been noted and will be important during selection of elite lines. Plant height and uniformity also show a whole range records and scores. Shorter and uniform varieties are more favourable for mechanisation during production. Plant height reaching 110 cm and above are unfavourable in hybrids. The foreign female lines are both above 110 cm whereas the male line is much shorter and more ideal. Uniformity of plants is important where production of cultivars will involve mechanisation. Landraces and wild types are mostly non-uniform and hybrids are generally bred for uniformity. Leaf length,

Table 3. Table of means for the eleven morphological variables.

Lines	DAYF	HD	LL	LW	PL	ND	LDG	NL	PLT	SD	UNIF
1. RP953-29-1	64	7.2	14.8	14.1	10.8	4	5	28.8	89.8	4.48	2
2. RHA 279	66	7.5	12.6	10.76	8.76	4	1	20.4	87.6	4.2	4
3. RHA 294	66	7.4	12.9	10.54	9.2	3	1	25.6	100.4	4.7	3
4. RHA340	75	8.4	13.2	13.2	9.9	2	1	29.4	101.2	4.9	2
5. RHA 344	68	6.6	11.9	10.66	8.3	4	1	23.8	100	4.52	4
6. RHA 363	64	9.3	12.4	11	8.28	4	1	24.2	97.6	4.9	3
7. RHA 368	64	7.3	9.98	8.96	8.64	2	1	28	94.6	3.76	3
8. RHA309	64	8.1	11.5	10.68	8.36	4	1	26.8	90	3.86	3
9. RHA 857	66	7.8	14	12.16	11.3	3	1	23.8	95.4	4.3	3
10. RHA 297	65	9.26	12.6	10.6	11	4	5	24.6	100	4.72	2
11. AP996-12-1-3-3	79	8.3	11.2	9.6	9.6	2	1	32.8	114.6	5.2	4
12. H55-9-3-1A	78	9.5	10.6	8	6.4	1	1	30.8	128.4	4.5	1
13. H55-2-2A	76	9.5	10.5	8.6	6.4	1	1	30.8	126.4	4	1
14. AP822-4-2-2-1	84	9.8	12	10.8	9.6	4	1	30.4	119.6	4.4	2
15. CMS HA 236	84	7.8	9.9	9.1	8.1	3	1	27.2	129.2	3.4	1
16. 497-74-9-4-2A	57	8	12.8	10.94	5.9	2	1	21.6	98.6	3.9	1
17. CMS124XIssanka	58	5.9	11.2	8.64	6.1	2	1	25.6	78	3	4
18. 79-75-7-2 A	64	8.8	13.5	10.9	8.4	3	5	31.8	95	4.96	3
19. CMS124XOroka	66	9.1	11.2	8.8	7.4	2	4	30	111.2	3.4	2
20. CMS HA 290	66	11.1	14.4	13.of4	11.1	5	1	23.8	112.4	5.4	2
21. CMS HA 89	87	9.8	13.7	12.5	11.5	2	1	29.4	101.8	5.4	1
22. CMS124 X HS20	60	8.8	11.3	10.04	7.56	3	1	27.4	85.4	4.8	3
23. 808-73-9-1A	55	10.4	11.2	9.7	8.4	2	3	23.6	105.6	3.4	3
24. 807-73-9-10A	55	10.6	13.5	11.7	7.6	2	3	22.8	101.8	3.6	2
25. CMS228	64	7.9	12.5	10.78	8	3	5	36.6	116	4.78	2
26. 807-73-11-10-2-2	54	9.06	11.8	11.4	5.56	5	1	23.6	77.76	4.52	1
Grand mean	67.55	8.68	12.05	10.61	8.36	2.76	1.93	27.16	104.29	4.282	2.24
St error	0.000	1.028	1.069	1.049	1.068	0.000	0.000	1.575	5.455	0.443	0.000

Lodging score (LDG);Number of leaves (NL);Nodding (ND);Head diameter (HD);Petiole length (PL);Leaf width (LW);Leaf length (LL); Stem diameter (SD);Plant height (PHT);Days 50% flower (DAYF); Uniformity score (U).

width and petiole length have implication on leaf size.Leaf size need not be too large as they may droop and reduce photosynthetic efficiency. Other variables like seed yield and oil content will have to be assessed in future studies under agronomic characterisation.

Hierarchical cluster analysis

Hierarchical cluster analysis in genstat discovery generated a tree diagram in Figure 1, for nearest neighbour analysis. Clustering indicated an overall similarity index of 91%. Which means the collection is 9% diverse. In general, an organism has maximum similarity of unit or 100% to itself and a dissimilarity coefficient of zero. According to the results of hierarchical clustering the lines are more similar amongst by 91% themselves than diverse. It seems the collection was distinctly grouped according to whether they are CMS or R lines by cluster analysis. According to the recorded variables, all of the R lines have been grouped together except RHA 297 and RP953-29-1 which is a recent introduction from outside Zimbabwe. The eight R lines show a similarity index of 96% meaning that they are 4% diverse. This might imply that they are a lot of similar lines or duplications in the R line collection. The CMS lines in this study are more diverse than the R lines, 91% similarity. About five clusters can be seen on the diagram. CMS HA 290 is the most distant from the rest of the lines, 9% genetic distance from the next neighbour. H55-2-2-A and H55-9-3-1A are the most similar with a 99% similarity index, more or less like a duplication.

Principal components analysis

PCA converted the variables to two vectors (Table 4) were used to plot a distribution of the lines on a 2-D scale. In essence the spread of the lines is observed as a scatter diagram (Figure 2). The latent vectors were plotted

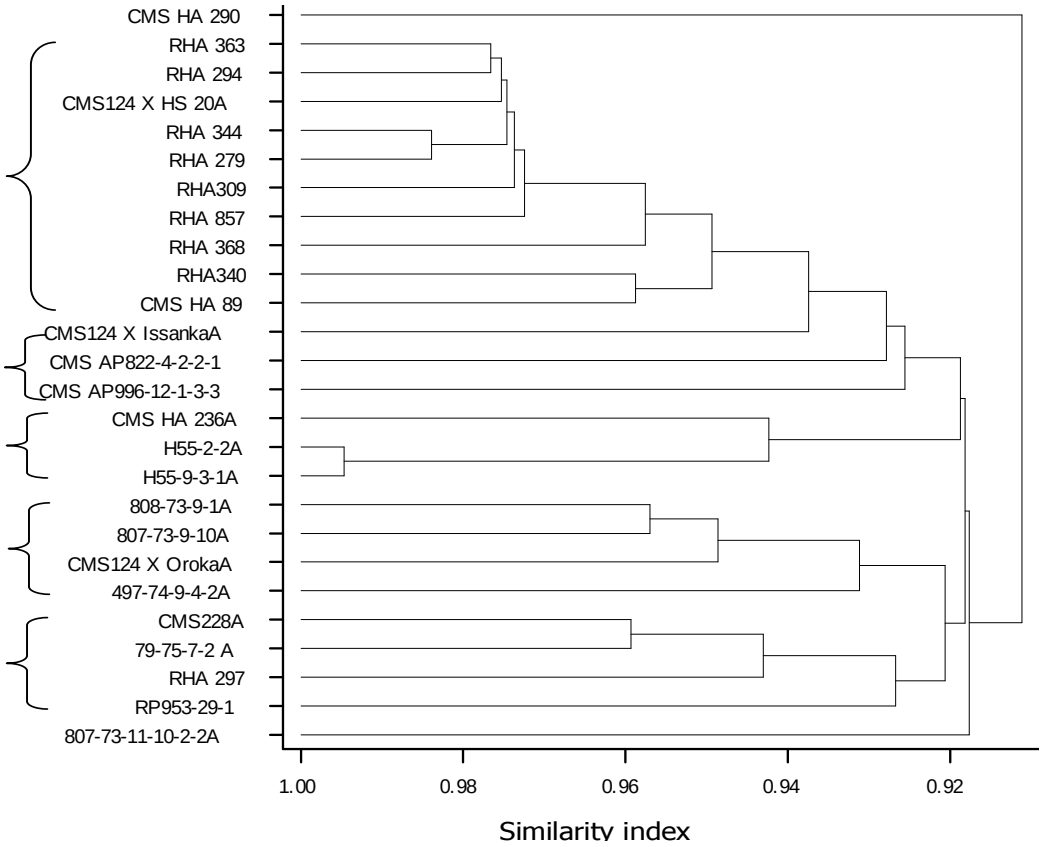

Figure 1. A cluster diagram generated after hierarchical cluster analysis of the means for the eleven morphological variables.

Table 4. List of latent vectors generated for principal components analysis.

Vectors	1	2
FLR	-0.10130	-0.47611
HD	0.06629	-0.31374
LL	0.49385	-0.06366
LODG	0.12705	-0.06092
LW	0.50541	-0.07779
NOD	0.36860	0.13357
NO LVS	-0.15432	-0.38744
PET L	0.37575	-0.24507
PLTHT	-0.23888	-0.47303
STDIA	0.32751	-0.31091
U	0.06838	0.33562

on a 2- D plane, resulting in a scatter diagram in Figure 2. The scatter diagram groups the lines into two groups, one big group, encompassing nineteen lines that is all of the R lines and nine CMS lines. The other smaller one, encompasses the seven remaining CMS lines. Clustering indicated an overall similarity index of 91%. It can be observed that the CMS lines are more spatially distributed as compared to the R-lines.

Generally globally, the genetic diversity of sunflower has a narrow genetic base and this is the major bottleneck in further improving the yields in sunflower (Yadava et al., 2012). This may be attributed to the fact

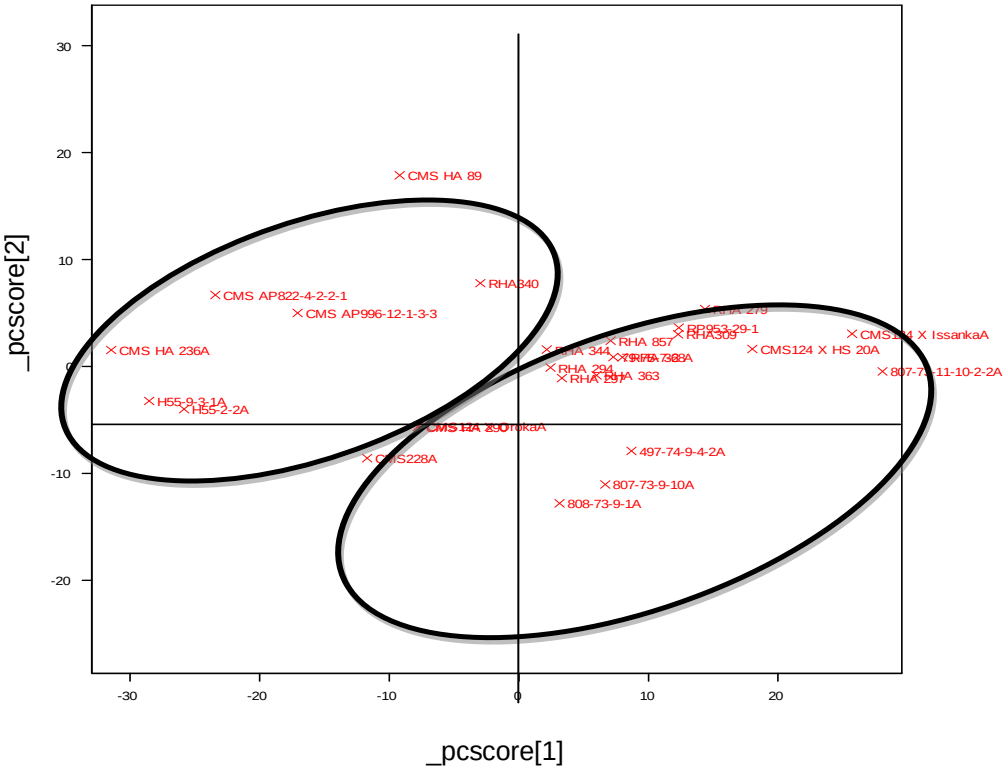

Figure 2. A scatter diagram generated after principal components analysis of the eleven morphological variables.

that all germplasm at some point have to be introgressed to a source of a CMS, R trait or any other fertilisation control trait. There is need to improve the diversity of the parental lines to achieve the higher level of heterosis in sunflower. Diversification of male sterility source may also help in improving stability of the hybrids. Similar work carried out by Kholghi et al. (2011) on diversity analysis in sunflower also reflects a limited germplasm collection. The units used in their study are not on a scale of 0 to 1 for genetic distance, but Euclidian genetic distance. However, the observations were a narrow genetic base in the collection they studied.

This is not like other crops, for example cowpea or the common bean. A similar study was carried out on *Phaseolus vulgaris* or the common bean, where thirteen quantitative and qualitative traits were analyzed using morphological established for *P.* vulgaris. The variables, pod length (cm), pod width (mm), pod beak length (mm), seeds per pod, seed length (mm), seed width (mm), seed height (mm), seed weight (g), pod color, color of wings, seed coat patterns, seed coat lighter color and seed shape. Genetic diversity observed was well over 80%.

Conclusion

In conclusion there is need for diversification of the collection from the national breeding institution otherwise inter-crosses will not produce much gain as they are almost similar to inbreeding. Inter-crosses should be carried out on the diverse material not on the duplicates if gains are to be realised. The three new foreign lines do not significantly diversify the collection. There is need for continued acquisition to enlarge the genetic base.

Conflict of Interest

The authors have not declared any conflict of interest.

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