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# Phenomic analyses of Indian and exotic accessions of Sesame (*Sesamum indicum* L.)

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A phenomic approach was undertaken to characterize seventy one accessions of sesame (*Sesamum indicum* L.) procured from India, Venezuela and USDA following the descriptor of NBPGR and IPGRI. The phenotypic traits varied extensively and the degree of polymorphism was highest in case of seed coat colour while few traits exhibited only binary type character state. Morphological observation revealed Venezuelan genotypes as the most derived ones while the Indian accessions, be it under NBPGR or USDA repository possess some of the wild character state of the traits. The hierarchical axial representation of phenogram indicated that one Indian accession under USDA repository (USIN06) was unique while the Venezuelan genotypes fell in a common cluster in neighbour-joining WPGMA tree. Principal coordinate analysis further resolved the relative distances between genotypes within each cluster/sub cluster. The traits related to trichomes of different plant parts showed highest correlation coefficients. A simple homology based algorithm; considering the character states as different colour codes and comparison of the codes of each genotype with that of a postulated 'target sesame' resulted in few Indian and exotic accessions of sesame on the basis of high 'scores', which will probably lead to a more precise means of selection of genotypes for future marker assisted breeding program.

Key words: Phenotypic traits, sesame accessions, phenogram, principal component analysis (PCA).

## INTRODUCTION

Sesame (Sesamum indicum L.) is an age-old yet underexploited oil seed crop (Laurentin and Karlovsky, 2006). Sesame breeding techniques vary greatly and have evolved from simple plant selection (Kinman and Martin, 1954) to hybrid cultivar development (Quijada and Layrisse, 1995). Revitalization of sesame-breeding methods assisted by marker assisted selection (MAS) could be of great value in breeding superior varieties. Although, sesame is one of the oldest cultivated plants in the world, its production and extension has been limited, particularly because of its low yield (Baydar, 2005). A major contributing factor to low yield in sesame is less emphasis on researches related to yield structure as a basis for progress in sesame breeding. Furthermore, sesame is a typically neglected crop since it is not studied by any of the international agricultural research centers; and the paradigm of sesame parallels many minor crops: sesame is not a major crop because there is

little research and other way around there is little research on sesame because it is not a major crop. Of the many important concerns where there remains scope for improvement to obtain a superior plant type of sesame, the most essential one is to accurately determine the time of seed maturity for harvesting. Due to inherent indeterminate nature of sesame the mature capsules near the base of the plant shatter resulting in loss of seeds while flowering continues on the top of the plant. Determinate growth habit in sesame, hence, offers the solution to such difficulties and it is now one of the prerequisites for adapting sesame to modern farming systems. This trait permits synchronized flowering and improved lodging resistance with shorter plant stature. However, on the other hand, in the determinate plant type yield is low compared to indeterminate counterparts (Uzun and Cagirgan, 2009). It is therefore important to improve the determinate types using both conventional and molecular plant breeding strategies. Apart from that, multiple flowers per axil, which will eventually lead to more number of pods - will be an added factor for improving yield of sesame. These traits however, are not

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very common in the available Indian sesame germplasm. These traits mentioned above and many more need to be picked right from the natural population and germplasm bank since it is always better to look for the stable natural variant than to forcefully create artificial variant. Marker assisted selection (MAS) and marker assisted breeding (MAB) are probably the most apt way to look and screen for the desirable genotypes and the present work, hence, aims to characterize the available national and international sesame germplasm with a futuristic approach to improve cultivated sesame, particularly in Indian context.

Since the essential prerequisite of MAS is characterization of the available germplasms through phenotyping and subsequently genotyping, the present study was designed with following objectives: (1) Characterization of the available sesame germplasm (both national and international) following the descriptor of National Bureau of Plant Genetic Resources. India (NBPGR) and International Plant Genetic Resources Institute (IPGRI) with a phenomic approach; (2) Use of the phenomic data set to understand the interrelationship between Indian/exotic genotypes by cluster analysis and phenogram construction; (3) Understanding the association between phenotypic traits by correlation analysis; and (4) Development of a simple homology based algorithm to match the available genotypes of sesame with the 'target' sesame, which has been envisaged on the basis of desirable combination of traits, selected either naturally or artificially. The present endeavor will probably lead to a more precise means of selection of genotypes for future marker assisted breeding program.

#### MATERIALS AND METHODS

Seventy one germplasm (seeds) of sesame were procured mainly from three sources (Table 1): (1) Thirty Indian accessions were obtained from National Bureau of Plant Genetic Resources, India (NBPGR); with this thirty, one locally cultivated variety (Tilottoma) was added as the source of Indian germplasm to make it thirty one. During procurement, care was taken so that the collection reflects the agro-climatic diversity of India; hence the collection represented thirteen sesame growing states of India. (2) Ten Venezuelan varieties were kindly provided by Dr. Hernán E Laurentin, Biologic Faculty, Sciences Department. Agronomy Universidad Centroccidental Lisandro Alvarado, Barquisimeto, Venezuela. (3) Thirty USDA accessions were obtained through Dr. Edward J (Ned) Garvey, Plant Germplasm Quarantine Centre, BARC-East, Beltsville, Maryland, United States. Of the USDA maintained germplasm, accessions originated in different countries (following the origin and diversity areas of sesame) were considered and it comprised of collections from Turkey, India (in USDA repository), China, Egypt, Iraq, Russia and Myanmar.

#### Descriptor and traits

Standard descriptor of sesame (NBPGR and IPGRI) was followed to arrive at thirty qualitative (phenotypic) traits and numeric was assigned for each and every character states under study.

#### Construction of phenogram and PCA

The seventy one germplasm under study were characterised on the basis of field data of three successive seasons and the table of character states was developed on the basis of mean data. The table of character state was imported to dedicated software and subsequent analysis was done considering continuous dissimilarity data to perform weighted neighbor-joining tree (WPGMA, graphical representation of cluster analysis) based on Euclidean distance calculation (with Bootstrapping) and Hierarchical tree with the help of software, DARwin 5.0.128. The clusters containing different genotypes with close proximity were further subjected to Principal Coordinate Analysis using the software NTSYSpc 2.02e to further determine the relation between distances of the genotypes present in each cluster. The values of Eigenvectors of first three PC components were taken in consideration in further data analysis (Sneath and Sokal, 1973).

#### Correlation between traits

The same data matrix of character state after necessary transposition (putting the column values into rows and vice versa) was used for correlation analysis between traits (Table 2). The analysis was done using statistical software MINITAB Release 13 Windows NT 4 © 2000 Minitab, Inc.

#### Phenomic table construction

Prior construction of phenomic table the traits were placed linearly one after one. Relative position of the traits was deduced from the correlation values. The pair of trait showing highest correlation value (Table 2) was placed together, and then it was followed with traits showing next higher correlation values and this continued up to the values of at least 5% (p<0.05) level of significance.

Subsequently, colour code was assigned to character states to obtain a 'Hit-map' type representation and altogether ten colour grades were used to denote degree of polymorphism of the traits. It ranged maximum from ten (in case of seed coat colour, the trait showing maximum variation) to minimum two (traits, which showing binary type qualitative representation e.g. stem shape in cross section, presence of leaf gland, colour of lower lip of petal, arrangement of capsule and shape of seed). The other traits were categorised in between number of colour grades depending upon their degree of Polymorphism (Figure 1a). Consequently, the character states of the traits of all the genotypes under study were assigned with specific colour grades resulting in a 'Hit-map' representation of the genotypes vs. traits (Figure 1b).

# Determination of percent similarity value towards 'target' Sesame

The graphical version of 'target' sesame with ideal depiction of colour code was conceptualized and the hypothesis was the right combination of character state of traits; which one are favoured by nature and which are the outcome of human (artificial) selection. After considering the right character state in case of both the selection, the colour grade of 'target' sesame was hypothesized taking in account the presence of both wild and derived character states so that the future sesame breeding programme is benefited due to the presence of easily scorable phenotypic marker(s) in the recombinants (Figure 1a, b). The 'target sesame' was envisaged as a moderately tall, basally branched plant type of short duration

 Table 1. Sesame germplasm under study.

Indian accessions of Sesamum indicum								
Variety/Accession No.	No. assigned	Abbreviation used	State					
IC 131569	1	NBM01	Maharashtra					
IC 131577	2	NBM02	Maharashtra					
IC 131578	3	NBM03	Maharashtra					
IC 131639	4	NBM04	Maharashtra					
IC 131700	5	NBM05	Maharashtra					
IC 131723	6	NBM06	Maharashtra					
IC 131726	7	NBM07	Maharashtra					
IC 131734	8	NBM08	Maharashtra					
IC 413217	9	NBAP01	Andhra Pradesh					
IC 413234	10	NBAP02	Andhra Pradesh					
IC 546218	11	NBAP03	Andhra Pradesh					
IC 413231	12	NBK01	Karnataka					
IC 132198	13	NBK02	Karnataka					
IC 131992	14	NBMP01	Madhya Pradesh					
IC 131989	15	NBMP02	Madhya Pradesh					
IC 131997	16	NBMP03	Madhya Pradesh					
IC 132000	17	NBMP04	Madhya Pradesh					
IC 131860	18	NBR01	Rajasthan					
IC 131987	19	NBR02	Rajasthan					
IC 131988	20	NBR03	Rajasthan					
IC 132084	21	NBR04	Rajasthan					
IC 131710	22	NBA01	Assam					
IC 131712	23	NBA02	Assam					
IC 131713	24	NBA03	Assam					
IC 96038	25	NBUP01	Uttar Pradesh					
IC 131630	26	NBG01	Gujarat					
IC 132250	27	NBB01	Bihar					
IC 132114	28	NBP01	Punjab					
IC 96073	29	NBHP01	Himachal Pradesh					
IC 131690	30	NBTN01	Tamil Nadu					
Tilottoma	71		West Bengal					
	Evotio appagaione of S	oomum indiaum						
Variety/Accession No	No accident	Abbreviation used	Country					
A3v32	31	VNI01	Venezuela					
	32	VN01	Venezuela					
	33	VNO2	Venezuela					
	34	VN03	Venezuela					
	35	VN0 <del>4</del> VN05	Venezuela					
	36	VN05	Venezuela					
	37		Venezuela					
FONLICEA	38	VN07	Venezuela					
	39	VN09	Venezuela					
	<u>4</u> 0	VN10	Venezuela					
PI 170714	41	UST01	Turkey					
PI 170725	42	UST02	Turkey					
PI 170726	43	UST02	Turkey					
PI 170738	44	UST04	Turkev					
PI 170748	45	UST05	Turkev					
PI 170753	46	UST06	Turkey					

Table 1. Con	itd
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PI 170758	47	UST07	Turkey	
PI 174353	48	UST08	Turkey	
PI 175907	49	UST09	Turkey	
PI 170730	67	UST12	Turkey	
PI 170733	68	UST13	Turkey	
PI 170743	69	UST14	Turkey	
PI 170763	70	UST15	Turkey	
PI 173952	50	USIN01	India	
PI 173956	51	USIN02	India	
PI 173960	52	USIN03	India	
PI 173962	53	USIN04	India	
PI 174952	54	USIN05	India	
PI 175305	55	USIN06	India	
PI 195122	56	USC02	China	
PI 195123	57	USC03	China	
PI 198155	58	USE01	Egypt	
PI 200105	59	USE02	Egypt	
PI 200108	60	USE03	Egypt	
PI 198156	61	USIR01	Iraq	
PI 198157	62	USIR02	Iraq	
PI 198158	63	USSU01	Russia	
PI 200109	64	USSU02	Russia	
PI 200106	65	USM01	Myanmar	
PI 200107	66	USM02	Myanmar	

nature containing more number of flowers / pods per axil with uniform maturity of pods and seeds of lighter testa colour. Furthermore, it would have the unique assemblage of traits like trichomes in different plant parts and visually identifiable phenotypic marker in flower petals.

To obtain the percent similarity of the genotypes under study with respect to the 'target' sesame, the character state(s) hypothesized to be present in an ideal condition, (that is, the 'target' sesame) were subsequently assigned 'scores': the concept was that if any trait of the genotypes under study matched with that of 'target' sesame the score would be 10, the next match would get 9 and the score would decrease accordingly. Following this rule each genotype would obtain its respective 'scores'. Apart from this formula, 'score' was calculated also on exact match of the character state of the traits between 'test' genotype and 'target sesame' and each match was assigned a score of 10. The mean values of these two calculations for each and every genotype under study were finally represented as 'percent similarity value towards target sesame'.

#### RESULTS

#### Phenotypic trait following descriptor

The phenotypic traits varied extensively and the degree of polymorphism was highest in case of seed coat colour where it varied from white to black with almost all possible in between colour grades (Figures 2a to c). Of the nine genotypes, which showed darkest seed coat colour, six belonged to Indian genotypes, four of which are in USDA repository and two in Indian (NBPGR) germplasm collection. Fifty percent of Venezuelan genotypes showed lightest seed coat colour character state. The basal branching character state was the most common one as it was noted in sixty five of the seventy one genotypes under study. The rest six either showed top branching or unbranched character state. The trait associated with trichome was stem/leaf/petiole/calyx/ corolla/capsule hairiness and it was represented by glabrous (no trichome)/sparse/hairy character state. The genotypes showing hairy phenotypes showed almost similar trend in all plant parts. Two Indian accessions (USIN02 and USIN06) under USDA repository showed maximum hairy phenotypes.

The bilabiate yet fused corolla of sesame showed range of variation in its all traits like exterior and interior corolla colour, corolla interior pigmentation, lower lip colour and presence or absence of Foveola (Small V or W shaped depression in the interior middle of the corolla below the lower lip) (Figures 2d to g). Though there were complete white flower coloration in certain genotypes under study but in most cases the genotypes were represented with conspicuous pigmentation mostly in the interior corolla (USIN06), lower lip (NBAP02) and few in exterior corolla (NBUP01, NBB01, NBP01, USIN05, USIN06) as well. Two associated traits of flowering, 
 Table 2. Correlation analysis between traits.

	PI height	Br habit	Stem tri	Stem CS	Leaf tri	Phyllo	Leaf gl	Leaf ang	Pet color	Pet tri	Day flow	50% flow	Flow p axl	Cal tri
Br habit	0.993													
Stem tri	0.435	0.464												
Stem CS	0.994	1	0.461											
Leaf tri	0.265	0.294	0.949	0.291										
Phyllo	0.288	0.314	0.896	0.309	0.945									
Leaf gl	0.264	0.293	0.948	0.29	1	0.945								
Leaf ang	0.268	0.296	0.95	0.293	1	0.945	1							
Pet color	0.265	0.294	0.948	0.291	1	0.945	1	1						
Pet tri	0.265	0.294	0.948	0.291	1	0.945	1	1	1					
Day flow	0.588	0.592	0.286	0.592	0.284	0.303	0.284	0.284	0.284	0.284				
50% flow	0.288	0.315	0.091	0.312	0.093	0.105	0.09	0.088	0.093	0.094	0.573			
Flow p axl	0.559	0.562	0.258	0.562	0.273	0.29	0.273	0.273	0.273	0.273	0.991	0.561		
Cal tri	0.218	0.257	0.852	0.253	0.902	0.852	0.902	0.903	0.902	0.902	0.255	0.072	0.256	
Corol tri	0.219	0.258	0.852	0.254	0.902	0.852	0.902	0.903	0.903	0.902	0.255	0.073	0.256	1
Ex cor col	0.219	0.258	0.852	0.254	0.902	0.852	0.902	0.903	0.903	0.902	0.255	0.072	0.256	1
In cor col	0.22	0.259	0.853	0.255	0.903	0.852	0.902	0.903	0.903	0.902	0.255	0.073	0.256	1
Co in pig	0.225	0.263	0.854	0.259	0.902	0.852	0.902	0.903	0.902	0.902	0.255	0.068	0.256	0.999
Low lip col	0.222	0.261	0.853	0.256	0.903	0.852	0.902	0.903	0.903	0.902	0.255	0.07	0.256	1
Foveola	0.222	0.261	0.853	0.257	0.902	0.852	0.902	0.903	0.903	0.902	0.255	0.07	0.256	1
Cap p pl	0.547	0.556	0.372	0.556	0.225	0.24	0.221	0.224	0.225	0.227	0.508	0.559	0.481	0.193
Cap shape	0.669	0.691	0.402	0.69	0.287	0.305	0.282	0.284	0.285	0.289	0.422	0.445	0.382	0.244
Cap arr	0.563	0.567	0.262	0.567	0.277	0.293	0.277	0.277	0.277	0.277	0.993	0.565	0.999	0.256
Cap tri	0.669	0.691	0.405	0.69	0.29	0.309	0.285	0.287	0.287	0.291	0.422	0.446	0.383	0.246
Cap length	0.403	0.386	0.104	0.382	0.035	0.057	0.032	0.032	0.034	0.038	0.296	0.282	0.215	-0.009
Cap col	0.403	0.386	0.108	0.382	0.039	0.059	0.036	0.036	0.038	0.042	0.296	0.281	0.215	-0.005
Cap dehsc	0.403	0.386	0.107	0.382	0.038	0.059	0.035	0.035	0.037	0.041	0.296	0.281	0.215	-0.006
Sd c text	0.151	0.127	-0.003	0.13	0.026	0.029	0.028	0.026	0.028	0.026	-0.009	-0.063	-0.009	0.021
Sd c col	-0.069	-0.047	0.369	-0.048	0.381	0.392	0.379	0.379	0.381	0.38	-0.35	0.042	-0.048	0.372
Sd shape	-0.099	-0.11	0.003	-0.105	0.049	0.017	0.05	0.044	0.047	0.046	-0.142	-0.243	-0.152	-0.043
Corol tri	Ex cor col	In cor col	Co in pig	Low lip col	Foveola	Cap p pl	Cap shape	Cap arr	Cap tri	Cap length	Cap col	Cap dehsc	Sd c text	Sd c col
1				•							•	•		
1	1													
0.999	1	1												
1	1	1	1											
1	1	1	1	1										

Table	2.	Contd.
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0.193	0.195	0.195	0.196	0.194	0.195									
0.244	0.244	0.245	0.242	0.242	0.244	0.579								
0.256	0.256	0.256	0.255	0.256	0.256	0.486	0.39							
0.246	0.246	0.246	0.244	0.244	0.246	0.58	1	0.39						
-0.008	-0.008	-0.009	-0.008	-0.008	-0.008	0.296	0.561	0.22	0.566					
-0.004	-0.005	-0.006	-0.004	-0.004	-0.004	0.297	0.561	0.22	0.567	1				
-0.005	-0.006	-0.007	-0.006	-0.005	-0.005	0.297	0.561	0.22	0.566	1	1			
0.022	0.024	0.021	0.027	0.022	0.02	-0.008	0.015	-0.008	0.013	0.06	0.058	0.06		
0.371	0.372	0.371	0.374	0.371	0.37	0.25	0.172	-0.044	0.172	-0.129	-0.129	-0.129	-0.02	
-0.041	-0.042	-0.044	-0.044	-0.042	-0.042	-0.172	-0.051	-0.15	-0.052	0.119	0.12	0.12	-0.03	-0.003

that is, days of flower initiation and days of 50% flowering showed wide range of variation from early, mid- to late flowering genotypes (Figures 2h to i). Of the five early flowering genotypes, three were NBPGR accessions (NBM03, NBM04, NBM06) while two were USDA accessions (UST04 and USIR01). All the ten genotypes from Venezuela showed late flowering (sixty days or bevond from the date of sowing). However, the NBPGR accession from Andhra Pradesh (NBAP02) showed very late flowering (>67 d). In general, sesame genotypes showed single flower per axil and consequently mono capsular arrangement (Figure 2m) at maturity but few showed two or more numbered flowers per axil and multiple capsules. Of these later types, all the ten were exotic, three being Venezuelan (VN03, VN05, VN10) and the rest seven were from USDA accessions; two originally from Turkey (UST09, UST13), two from China (USC02, USC03), and one each from Egypt (USE01), Soviet Union (USSU01) and Myanmar (USM01). Of all multi capsular plant types, USC02 was most significant since it showed 5-6 capsules per axil in most of the cases (Figures 2k and n). The trait capsule dehiscence was represented in sixty-nine out of the seventy one genotypes by three character

states in the descriptor, viz. non-shattering, partially shattering and completely shattering.

Some of the representative phenotypic traits of the sesame genotypes: Polymorphism of seed coat colour - (a) white seeded UCLA1. (b) brown seeded NBM01, (c) black seeded USIN04; polymorphism of interior and exterior corolla pigmentation - (d) white flowers of NBM01, (e) pink lip of USIN01, (f) coloured foveola of NBAP02, (g) profusely hairy and pigmented flower of USIN06; (h) early flowering NBM07, (i) late flowering NBAP02 (x-axis: days after sowing; yaxis: number of plants per plot (genotype) showing flowering; intercept to the x-axis denotes days to 50% flowering); (j) repetitive flowering from axil in USIR02; (k) multiple pods per axil in USC02; (I) hairy pods in USC03; (m) single pod per axil NBAP03; (n) multiple pods per axil in USC02.

One genotype under study showed completely shattering capsule types while only two (UST03 and USIR02) exhibited partially shattering capsules. Degree of polymorphism was more in plant height and the locally cultivated Indian variety (Tilottoma) along with two Venezuelan genotypes (VN03 and VN05) showed highest plant height, while seven accessions from USDA of diverse country-origin showed very short plant types. Seed coat texture was another trait that showed a wide range of variation and the genotypes revealed all sort of variation with smooth testa at one end to the reticulate rough texture to the other extremity.

#### Phenogram and PCA

The hierarchical axial representation of the phenogram demarcated the seventy one genotypes under study into a unique pattern. Sixty six of seventy one clubbed into a common node (number 100) with different tiers among themselves while rest five genotypes showed distinctness. Of these five, four (NBR01, NBM08, NBR04, Tilottoma) were placed in one side of the phenogram while the relative position of USIN06 was totally on the opposite side of the 'tree' (Figure 3).

A clearer grouping of the genotypes was obtained in the neighbour-joining (WPGMA) form of tree representation where the genotypes were apparently separable into six sub clusters (Figure 4). The genotypes, essentially formed three clusters, of which cluster 3 was represented by





Figure 1. (a). The colour grades assigned to character states of the traits depending upon their degree of polymorphism. (b). The 'hit map' representation of the genotypes under study following the colour grades.



Figure 1. (a). The colour grades assigned to character states of the traits depending upon their degree of polymorphism. (b). The 'hit map' representation of the genotypes under study following the colour grades (contd.).



**Figure 2.** Some of the representative phenotypic traits of the sesame genotypes: Polymorphism of seed coat colour - (a) white seeded UCLA1, (b) brown seeded NBM01, (c) black seeded USIN04; polymorphism of interior and exterior corolla pigmentation – (d) white flowers of NBM01, (e) pink lip of USIN01, (f) coloured foveola of NBAP02, (g) profusely hairy and pigmented flower of USIN06; (h) early flowering NBM07, (i) late flowering NBAP02 (x-axis: days after sowing; y-axis: number of plants per plot (genotype) showing flowering; intercept to the x-axis denotes days to 50% flowering); (j) repetitive flowering from axil in USIR02; (k) multiple pods per axil in USC02; (l) hairy pods in USC03; (m) single pod per axil NBAP03; (n) multiple pods per axil in USC02.



Figure 2. Contd.



Figure 3. The hierarchical axial representation of phenogram of seventy one genotypes constructed with software DARwin 5.0.128.

the least number of genotypes (six) under one cluster and this comprised all of exotic accessions under USDA repository. Of these six, two belonged to China (USC02 and USC03) and one each from Russia (USSU01), Turkey (UST09), Myanmar (USM01) and Egypt (USE01). NTSYSpc taking into consideration the data of these six genotypes revealed that the first PC axis accounted for 70% of the total multivariate variation while the second and third accounted for 10 and 6%, respectively (Figure 5 and Table 3).

Cluster 1 of the phenogram (Figure 4) comprised of three sub clusters, of which sub cluster 2 and 3 indicated a common origin (node number 139). The sub cluster 2 contained twelve genotypes, of which only three were exotic (under USDA repository, viz. UST14, UST15 and USE02) while rest nine were Indian accessions under NBPGR germplasm collection. NTSYSpc taking into consideration the data of these twelve genotypes revealed that the first PC axis accounted for 54.5% of the total multivariate variation while the second and third accounted for 7.5 and 7.2%, respectively (Figure 5). The sub cluster 3 showed the assemblage of thirteen genotypes, of which twelve were accessions from NBPGR of different states of India, while the rest though exotic (under USDA repository) but has an Indian origin

#### (USIN03).

NTSYSpc with the data of these thirteen genotypes revealed that the first PC axis accounted for 61% of the total multivariate variation while the second and third axes accounted for 6.5 and 6.3%, respectively (Figure 5). The first sub cluster of cluster 1 showed the relatedness of twelve genotypes, of which nine were from USDA repository, six of Turkish origin (UST01, UST02, UST04, UST05, UST06, and UST07), two from Iraq (USIR01, USIR02) and one from Egypt (USE03). Only three NBPGR accessions (NBM04, NBM05 and NBK01) from India shared similarities with the nine exotic genotypes. NTSYSpc of the data of these twelve genotypes revealed that the first PC axis accounted for 63% of the total multivariate variation while the second and third axes accounted for 6 and 5%, respectively (Figure 5).

Cluster 2 of the phenogram (Figure 4) comprised of two sub clusters, of which fifteen belonged to sub cluster 1 and thirteen to sub cluster 2. Among the fifteen genotypes of sub cluster 1, nine were found to be under USDA collection, while six belonged to NBPGR, India. Of the exotic nine, four were of Indian origin (USIN01, USIN04, USIN05 and USIN06), while two came from Turkey (UST12 and UST13) and one each from Russia (USSU02) and Myanmar (USM02). NTSYSpc taking into



Figure 4. The Neighbour-joining (WPGMA) representation of phenogram of seventy one genotypes constructed with software DARwin 5.0.128 showing clusters / sub clusters.

consideration the data of these fifteen genotypes revealed that the first PC axis accounted for 50% of the total multivariate variation while the second and third axes accounted for 8.9 and 8%, respectively (Figure 5). The sub cluster 2 of cluster 2 exhibited unique assemblage of all the ten Venezuelan genotypes along with two USDA collections (UST08 and USIN02). NBAP02 was the sole representative of Indian accession (NBPGR) in this sub cluster. NTSYSpc with the data of these thirteen genotypes revealed that the first PC axis accounted for 51% of the total multivariate variation while the second and third axes accounted for 9 and 7.8%, respectively (Figure 5).



Figure 5. Principal Coordinate Analysis of the individual cluster /sub cluster of Neighbour-joining phenogram (Figure 4) using the software NTSYSpc 2.02e.

#### **Correlation between traits**

Correlations among traits ranged from -0.3 to 100%, however, significant relationship (p<0.05) between characters were noted only for the positive correlations. Most of the correlated characters that suggest significant

linear relationship were in the range of 80 to 100%. The traits related to trichomes of different plant parts (both reproductive and vegetative) showed highest correlation coefficients. This was also true for the pigmentation traits. The only character that did not show any significant correlation with any of the other character was shape of

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S/N	Eigenvalue	Percent	Cumulative			
	Eigenvalues for clu	ster1 subcluster 1				
1	7.55328717	62.9441	62.9441			
2	0.75251137	6.2709	69.2150			
3	0.68737840	5.7282	74.9431			
4	0.58643645	4.8870	79.8301			
5	0.51349430	4.2791	84.1092			
6	0.50469997	4.2058	88.3151			
7	0.40282095	3.3568	91.6719			
8	0.34678460	2.8899	94.5618			
9	0.26631389	2.2193	96.7811			
10	0.16312223	1.3594	98.1404			
11	0.15658729	1.3049	99.4453			
12	0.06656338	0.5547	> 100%			
		atari aukaluatar 0				
4	Eigenvalues for clu		E4 EC00			
1	0.04729757	04.0000 7 5510	04.0000 60.1107			
2	0.90622063	7.0016	62.1127			
3	0.86779159	7.2310	09.3442 75.4004			
4	0.73790300	0.1492	75.4934			
5	0.5000/060	4.0723	00.1037			
6	0.01020701	4.2024	04.4101			
7	0.22274314	3.5229	01.1754			
0	0.30012752	3.2344	91.1754			
9	0.30363132	2.0021	93.7075			
10	0.29467022	2.4070	90.1047			
12	0.24361103	1.8052	> 100%			
	Eigenvalues for clu	ster1 subcluster 3				
1	7.94136592	61.0874	61.0874			
2	0.84972066	6.5363	67.6237			
3	0.81532521	6.2717	73.8955			
4	0.71991161	5.5378	79.4333			
5	0.58664414	4.5126	83.9459			
6	0.42902119	3.3002	87.2461			
/	0.36895036	2.8381	90.0841			
8	0.33063154	2.5433	92.6275			
9	0.29496195	2.2689	94.8964			
10	0.21/30944	1.6/16	96.5680			
11	0.1/4/0241	1.3439	97.9119			
12	0.15894186	1.2226	99.1345			
13	0.11251372	0.8655	100.0000			
	Eigenvalues for clu	ster 2 subcluster 1				
1	7.57560405	50.5040	50.5040			
2	1.34139037	8.9426	59.4466			
3	1.20901075	8.0601	67.5067			
4	0.84532975	5.6355	73.1422			
5	0.66894444	4.4596	77.6019			
6	0.61735467	4.1157	81.7176			
7	0.49066389	3.2711	84.9887			

 Table 3. Eigenvalues of NTSYSpc corresponding to Figure 5.

8	0.41062008	2.7375	87.7261
9	0.37433078	2.4955	90.2217
10	0.36182026	2.4121	92.6338
11	0.32003404	2.1336	94.7674
12	0.27615906	1.8411	96.6084
13	0.21506471	1.4338	98.0422
14	0.19266916	1.2845	99.3266
15	0.10100398	0.6734	> 100%
	Eigenvalues for clu	ster 2 subcluster 2	
1	6.70659367	51.5892	51.5892
2	1.18514169	9.1165	60.7057
3	1.01442770	7.8033	68.5089
4	0.82038052	6.3106	74.8196
5	0.65409121	5.0315	79.8510
6	0.50535079	3.8873	83.7384
7	0.48826457	3.7559	87.4942
8	0.40273439	3.0980	90.5922
9	0.37836066	2.9105	93.5027
10	0.29948110	2.3037	95.8064
11	0.23022291	1.7709	97.5773
12	0.16583785	1.2757	98.8530
13	0.14911292	1.1470	> 100%
	Eigenvalues	for cluster 3	
1	4.21415393	70.2359	70.2359
2	0.64355788	10.7260	80.9619
3	0.38706234	6.4510	87.4129
4	0.37543380	6.2572	93.6701
5	0.23377723	3.8963	97.5664
6	0.14601482	2.4336	100.0000

Table 3. Contd.

the seed (Table 2).

# Phenomic table and determination of percent similarity value towards 'target' Sesame

The desirable combination of character states of the twenty nine traits (excluding shape of seed) were envisaged taking in consideration of both natural and artificial (human) selection on the basis of simple logic and theory. For example un-branched type was considered to be the choice of nature while branched type was a resultant of human selection from the view point of greater yield. Of the two branching types, the basal branching morph is definitely desirable since the top branching type is prone to lodging. Trichomes of both vegetative and reproductive plant parts were considered as selection of nature whereas more glabrous types are ensuing upon artificial selection. Similarly, pigmentation in different plant parts and especially in seed coat was

considered to be nature's choice while the lighter shaded types are the derived character states. This is particularly true in seed coat since the seeds with darker colour shade often leave a stain in the processed oil. Considering these, the right combination of character states of the 'target sesame' was conceptualized and caution was made so that it reflects an ideal combination of 'wild' and 'derived' character states since the more hairy plant types or unique pigmentation in different floral parts often serve as 'phenotypic markers' for selection of recombinants (Figure 1a).

The colour code of the 'target sesame' was then matched with colour codes of all the genotypes under study and a specific value of each and every genotype was obtained on the basis of calculation as mentioned earlier. This value was denoted as 'percent similarity value towards target sesame' of every genotype and those were subsequently short-listed considering the 'cutoff' value of above 70%.

Of the thirty-one Indian sesame germplasm only nine

showed values greater than 70% and those are as follows: NBM02 (71.72%), NBM07 (76.89%), NBMP01 (71.20%), NBMP02 (75.00%), NBR01 (71.37%), NBR02 (71.55%), NBUP01 (70.86%), NBP01 (73.79%) and NBTN01 (71.37%). Three Venezuelan genotypes showed values greater than 70% and those are as follows: VN02 (83.44%), VN03 (81.03%) and VN05 (72.93%). Of the forty USDA accessions, nine showed values higher than 70%. Of these, three were originally from Turkey (UST13, 70.52%; UST14, 70.34% and UST15, 72.07%), two were from India (USIN02, 71.37%; and USIN06, 75%), one from China (USC02, 70.17%), one from Egypt (USE01, 74.48%), one from Iraq (USIR02, 72.06%) and one from Russia (USSU02, 70.34%).

## DISCUSSION

Sesame is a plant breeder's dream crop because it represents great genetic variability (Janick and Whipkey, 2002). Since any successful breeding programme depends on variability present among the genotypes (Banerjee and Kole, 2009), the present study with a futuristic approach of broadening the gene pool of Indian sesame was initiated procuring as many representative genotypes as possible both from Indian and International repositories. The results of analysis of phenotypic traits following the descriptor revealed an array of polymorphism in almost all the traits under study. The assemblage of traits - be it 'wild' or 'derived', was copious within all the accessions cutting across the barrier of geographical origin. The oldest remnants of sesame, found in the Harappa valley in the Indian subcontinent (Bedigian and Harlan, 1986), date the origin of these activities to at least 5500 BC. Since sesame growers have been manipulating the crop due to migration and trade for centuries it has caused a steady gene flow among different geographical areas. Of all the accessions under present study, the Venezuelan genotypes seemed to be the most improved ones since most of the desirable attributes for a high yielding variety like multiple flowering and pod development per node, longer flowering branches with near synchrony in pod development, seeds of lighter shades etc were characteristic features of those genotypes. The Indian accessions, both in NBPGR and USDA repositories showed traits like trichomes in different plant parts, unique pigmentation etc, which can effectively be used as morphological marker(s) in future breeding programme. Furthermore, low number of flower/pod per axil, indeterminate maturity and asynchrony of pod development along with shattering nature of pods at maturity of all the genotypes indicated further scope of improvement of these genotypes by marker associated breeding techniques.

The genotypes, when analyzed statistically on the basis of the different character states of these thirty traits, the hierarchical axial representation of the phenogram

indicated that one Indian accession under USDA repository (USIN06) was completely away from the 'root' of the 'phenetic tree'. Re visiting the morphological features of this accession revealed that the plant type was not only the shortest of all the genotypes, but it is also a profusely hairy one with unique pigmentation both in exterior and interior corolla along with its dull black seed coat colour. Precisely speaking, it seemed to be a perfect example of a genotype with assemblage of most of the 'wild' character states of the traits and the hierarchical 'tree' has clearly secluded this from all other genotypes. Contrary to this hierarchical approach, the neighbour-joining WPGMA analysis indicated clearer demarcation of the genotypes based on their overall similarity/dissimilarity. The smallest cluster consisting of all exotic accessions of USDA (USC02, USC03, USSU01, UST09, USM01, and USE01) is plausibly the end resultant of multiple flowers/pod per axil, a significant trait related to vield, which was omnipresent in all these genotypes. That all the desirable attributes have already been clubbed to a large extent in Venezuelan accessions, a general observation made earlier, was substantiated by their grouping in a close cluster (sub cluster 2 of cluster 2). The interesting co existence of NBAP02 within this cluster is due to its extremely late flowering nature (all the Venezuelan counterparts are also moderate to late flowering types). Apart from the aforesaid clusters, the others either showed predominant assemblage of either NBPGR (sub cluster 3 of cluster 1, sub cluster 2 of cluster 1) or USDA (sub cluster 1 of cluster 1) accessions. Sub cluster 1 of cluster 2, however, showed relatively proportionate association of NBPGR and USDA accessions. The relative 'distance' of Indian and exotic germplasm within each cluster/sub cluster was further resolved by principal coordinate analysis. A critical analysis of the Eigen values of NTSYSpc indicated the following genotypes as plausibly the 'unique' ones: USIR02 (sub cluster 1 of cluster 1), UST15 (sub cluster 2 of cluster 1), NBMP02 (sub cluster 3 of cluster 1), USIN05 and USIN06 (sub cluster 1 of cluster 2), USIN02 (sub cluster 2 of cluster 2) and USC02 (cluster 3). Though ecological and geographical factors seemed to have not played an important role in the evolution of sesame since no association was obtained between genetic diversity and accession origin through AFLP analysis (Laurentin and Karlovsky, 2006) but the present endeavor on the basis of phenetics of morphological parameters probably indicate that certain uniqueness of sesame gene pool is being still preserved in few genotypes of specific geographical region in spite of intense human activities.

The association between traits as revealed by correlation analysis indicated steady 'bondage' of few, having high correlation values. Though it is pre mature to state whether the traits as linked ones prior analyzing linkage of these in recombinants but two of the phenotypic markers, viz. trichomes and pigmentation, especially in the reproductive structures warrant further investigation.

Based on the correlation values between traits and simple logic, the phenomic table was constructed and the best possible combination of colour code of the 'target sesame' was postulated. By matching the codes of all the genotypes under study with the code of the 'target sesame' percent similarity value was derived. This attempt, probably the first of its kind, gave clear cut values to the genotypes, which led to short listing of few Indian and exotic accessions of sesame. This not only will help for planning more precise breeding strategy but also will facilitate looking for molecular marker specific for genotype and consequently phenotypic trait from these selected genotypes.

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