

GENETIC DIVERSITY IN *SESAMUM INDICUM* L.

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Abstract

Sesame (*Sesamum indicum* L.), is the most ancient and important oilseed crop for its good quality edible oil. Much variability among genotypes exists in sesame which helps the breeder to choose good parents in hybridization. In the present study, 30 genotypes were studied for 12 morphological traits. Correlation analysis exhibited that seed yield per plant was positive and significantly correlated with capsules per plant and 1000-seed weight. Apart from morphological data, initial genotyping was conducted for 32 microsatellites but finally only polymorphic primers were selected for genetic diversity study. Estimated polymorphism information content (PIC) value ranged from 0.07 to 0.87 and SSR 24 showed to be highest at 0.87. The dendrogram was constructed using the software NTSYS Pc Ver. 2.20 based on morphological and molecular data. Geographical diversity did not corroborate with genotypic diversity as the cluster composition revealed that genotypes belonging to different geographical origin grouped in the same cluster.

Introduction

Sesame (*Sesamum indicum* L.) is one of the oldest oil seed crops which is commonly known as *Til* or *Gingelli*. Sesame (*Sesamum indicum* L.) belonging to Pedaliaceae, is widely cultivated in tropics and subtropical area. During Harappan and Anatolian eras over 4,000 years ago sesame was cultivated and domesticated on the Indian subcontinent (Bedigian and Van der 2003). Due to the presence of its high seed oil content and the excellent qualities of the seed oil and meal, it is referred as “Queen of oil seed” (Fukuda *et al.* 1986). Sesame is a good source of oil (44 - 58%), protein (18 - 25%), and carbohydrates (13.5%) (Bedigian *et al.* 1985). Sesame seed contains 83 - 90% unsaturated fatty acids such as oleic acid (18: 1) (39.6%) and linoleic acid (18:2) (46.0%) and it has desirable physiological effects that include antioxidant activity and blood pressure and serum lipid lowering potential (Yermanos *et al.* 1972). Sesame oil exerts anti-cancer properties both *in vitro* and in animal bioassays due to presence of lignans (Fukuda *et al.* 1986). Sesame seeds are used in the preparation of number of food products and sweets confection. India is the second world leader with 1.9 million hectares area and ranks third in production (797700 tons) in the world (FAOSTAT 2016). India has produced 893000 tonnes in 2010 but after that the production of sesame has been declined in recent years. Improvement of sesame production is still hampered due to lack of promising variety with high yield. Assessment of genetic diversity is an initial step in breeding programme where large variability among potential parents is always desirable. Assessment of genetic diversity using agro-morphological and molecular markers is one of the effective methods for diversity analyses in sesame that can be used in future breeding program and also significant to improve sesame varieties. Diversity analyses based only on morphological characters are prone to environmental bias due to environmental influences and complex genetic structure of different morphological traits (Pandey *et al.* 2015). Several of agro-morphological trait based studies showed a high genetic diversity in sesame populations (Bisht *et al.* 1998, Arriel *et al.* 2007, Iqbal *et al.* 2016). Recently, quite a lot of molecular markers have

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been developed and already applied to sesame genotyping, such as RAPD (Ercan *et al.* 2004), AFLP (Ali *et al.* 2007 and Laurentin and Karlovsky 2006), ISSR (Kim *et al.* 2002) and expressed sequence tags-SSR (EST-SSR) (Wei *et al.* 2008). Microsatellites or simple sequence repeat has proved its unequivocal supremacy because of high abundance and reproducibility, easy scoring, extensive coverage and codominant nature (Dixit *et al.* 2005, Pandey *et al.* 2015). SSR markers are widely used for high-throughput genotyping and map construction for the candidate genes. Often EST-SSR is used for diversity study. The aims of the present study were to elucidate genetic relationships among 30 sesame genotypes specifically chosen from different origin representing diverse centres of origin across India and also one genotype each from USA and Bulgaria, so that the analysis would be meaningful for breeders for selecting desirable parents.

Materials and Methods

Thirty diverse genotypes were collected from different parts of West Bengal and some major sesame growing states of India along with one genotype from USA and Bulgaria (Table 1). Field experiment for agro-morphological study was carried out at Calcutta University Agricultural Farm, Baruipur, south 24 Proganas (22°51' latitude north and 88°25' longitude east) following RBD with 3 replications with spacing of 15 cm between plants and 40 cm between rows, during the pre-kharif season 2016 and 2017. Ten randomly selected plants from each variety were chosen and data were taken for 12 agro-morphological traits. The data were recorded and calculated the average values of 2 years.

Thirty diverse genotypes used were further analyzed using microsatellites or simple sequence repeat molecular marker. For genomic DNA extraction, young leaves of germinated seeds (7 - 10 days) were used. Using liquid nitrogen, 0.5 g of leaves was grounded and DNA was extracted by CTAB method (Saghai-Marooif *et al.* 1984). Initially using Primer 3 software, 32 genomic SSR markers were designed from sequenced "Rama" cultivar.

DNA amplification was performed in 25 µl reaction mixture containing 50 ng genomic DNA, 0.2 µmol/l SSR primers, 10X PCR buffer, 50 mM MgCl₂, 0.2 mM dNTPs and 0.5 units *Taq* DNA polymerase. The PCR amplification was performed using a Eppendorf thermo cycler (Germany), with the following cycle profiles: denaturation at 95°C for 5 min, in 38 cycles 30 seconds at 95°C, 45 sec at the annealing temperature depending on particular primer pair (~55°C), 1 min at 72°C (extension) and final extension at 72°C for 5 min. After completion of PCR, the amplicons were separated through electrophoresis in 2% agarose gel prepared with 1× TAE buffer at 120 volts and stained with ethidium bromide (Biorad, USA).

The size of amplified DNA was recognized by comparing the band with respect to the molecular weight of a DNA ladder (50 base pair DNA ladder, Bioline). On the basis of presence and absence of a specific sized allele, scoring was done using 1/0 matrix (the presence of an allele was denoted by 1 and absence similarly by 0) was recorded and also the amplified DNA according to their allelic size (basepairs) for all 30 genotypes. Polymorphism information content (PIC) values were calculated. Effective allele per locus (Aep) was computed by $1 / (1 - \text{Hep})$ as suggested by Weir (1990), where Hep designates the genetic diversity for each locus and Hep is equal to $1 - \sum P_i^2$, P_i means the frequency of i^{th} allele at the locus (Pandey *et al.* 2015). The construction of UPGMA-based dendrogram was carried out from the molecular allelic data of 30 genotypes with the help of software NTSYS Pc (Ver. 2.20).

Table 1. List of sesame genotypes for present experiment.

Sl. No.	Name of genotypes	Seed coat colour	Centre of origin	Year of release	Parentage
1	EC-90/EC-310448 (36)	Black	Bulgaria (exotic collection, NBPGR)	-	Exotic collections
2	Savitri	Light brown	West Bengal (PORS)	2008	Selection from germplasm SWB32
3	EC-103/EC-1649666(52)	Black	USA(exotic collection , NBPGR)	-	Exotic collections
4	Rama	Reddish brown	West Bengal (PORS)	1989	Selection from 'Khosla' local
5	IC59/IC-204063	Brown	Indigenous collection, NBPGR	-	Indigenous collection
6	CUMS-06	Brown	West Bengal (CU)	-	-
7	CUHY-57 (Pragnya)	Light brown seed	West Bengal (CU)	Identified by CVRC,	Uma × TKG 352
8	CUMS-17 (Suprava)	Light brown	West Bengal (CU)	2018	mutant of IC 625735
9	V 12	Brown	West Bengal, local land race	Notified by CVRC,	-
10	CUMS-20	Dark brown	West Bengal (CU)	2018	-
11	Tilottama	Blackish brown	West Bengal (PORS)	1984	Local landrace
12	CU-12	Dark brown	West Bengal (CU)	-	IC 21706 (0.5% EMS)
13	IC-64/IC-14053	Brown	Indigenous collection, NBPGR	-	Selection from local germplasm
14	Thilathara	Blackish brown	Kerala	2006	Jinardi Ducea2
15	JLT 408	White	Maharashtra	2010	OSC593 × NIC8316
16	RT-351	White	ARS, SKRAU, Mandore, Rajasthan	2010	Indigenous collection
17	GT-10	Black	Gujarat	2002	-
18	AT-306	White	Amreli, Gujarat	-	Padma × Yuzhi-8
19	HT-2	White	Haryana	2012	NIC 8409 × RT 127
20	OSC-593	Dark brown	Bhubaneswar , Odisha (OUAT)	2012	Selection from TNAUI7
21	TKG22	White	ZARS, JNKVV, Tikamgarh, MP	1995	-
22	Germplasm 80	White	Jabalpur, MP	-	-
23	AT-238	White	Amreli, Gujarat	-	-
24	DS-35	White	Dharwad, Karnataka	-	-
25	Prachi	Black	Odisha (OUAT)	2004	Local landrace
26	Shekhar	White	Uttar Pradesh	-	HT6 × JLT3
27	MT-75 (Pragati)	White	Uttar Pradesh	-	Local landrace
28	Nirmala	White	OUAT, Bhubaneswar, Odisha	2002	-
29	TKG-306	White	ZARS, JNKVV, Tikamgarh, MP	2003	Mutant of B67
30	TKG-308	White	ZARS, JNKVV, Tikamgarh, MP	2006	-
				2008	Mutant of B-67

Results and Discussion

Evaluation of genetic diversity in sesame provides an opportunity for selecting desirable parents to develop new and improved variety with desirable characteristics like high yield, high oil content and others traits. Twelve agro-morphological traits were recorded from the 30 genotypes of *Sesamum indicum* L. The genotypic coefficient of variation (42.36) and the phenotypic coefficient of variation (48.14) both were found to be highest for capsules per plant followed by seed yield per plant, primary branch per plant, secondary branch per plant (Table 2). Saxena and Bisen (2017) also observed the similar trend. The phenotypic coefficient of variation (PCV) was greater than GCV for all characters but both resembled other very closely which concluded that the environment has very little effect on the expression of these characters. The heritability estimate ranged between 28.94 to 78.21 % and was found to be high for days to maturity (78.21), followed by number of capsule per plant (77.41), plant height (61.49). High GCV coupled with high heritability can provide more desirable information than a single parameter alone. High GA occurs only due to additive gene action. So heritability estimate coupled with GA would be more useful than heritability alone. The correlation study revealed that strong positive significant correlation was present between yield per plant and capsules per plants and 1000 seed weight (Table 3). Similar observations were found by Uzun and Cagirgan (2001) and Iqbal *et al.* (2016). As the objective of a breeder is to improve the yield potential the present investigation may highlight that selection to improve the traits like capsule per plant and seed yield per plant will be judicious for upgrading productivity of the crops. The results confirm previous findings (Iqbal *et al.* 2016, Subramanian and Subramanian 1994). Thus selection for high number of capsules per plant and more seed weight in segregating generation would obviously help to develop promising high yielding lines.

Table 2. Estimates of genetic parameters.

Estimates	GCV (%)	PCV (%)	H (%)	GA
50% flowering	8.49	12.82	43.83	2.95
Plant height	16.41	20.93	61.49	23.32
Inter node length	11.22	14.55	59.44	0.56
Primary branch/plant	20.85	30.52	46.69	0.78
Secondary branch/plant	16.83	31.29	28.94	0.29
Capsules/plant	42.36	48.14	77.41	51.21
Capsule length	7.36	9.92	54.96	0.2
Capsule diameter	5.11	10.72	22.72	0.01
Days to maturity	5.81	6.57	78.21	8.63
Seeds/capsule	5.86	9.19	40.62	3.11
1000-seed weight	9.53	13.36	50.89	0.32
Seed yield/plant	32.96	47.81	47.52	2.74

GCV = Genotypic co-efficient of variation, PCV = Phenotypic co-efficient of variation, H = Heritability and GA = Genetic advance.

The agro-morphological trait based cluster analysis grouped the 30 sesame genotypes into five main groups (Fig. 1). Two exotic collections were placed in cluster I. The cluster II consisted of maximum number of genotypes (24) and cluster III contained two genotypes while cluster IV and cluster V comprised of single genotype. Agro-morphological data based genetic distance between 30 sesame genotypes ranged from 0.05 - 0.52 with a mean of 0.28 and the similar trend was

Table 3. Correlation coefficient for yield and yield related traits in 30 sesame genotypes,

	50% F	PH	I.L	PB/ Plant	S.B/Plant	CP	CL	CD	DM	SC	1000-SW	Yield/ plant
50% F	1											
PH	0.041	1										
IL	0.197	0.090	1									
PB/plant	-0.011	0.255	0.784**	1								
SB/plant	0.019	0.089	0.025	0.389*	1							
CP	0.264	0.297	0.290	0.379*	0.217	1						
CL	0.175	-0.191	0.133	0.167	0.039	0.082	1					
CD	-0.152	-0.207	-0.136	0.203	-0.248	0.021	0.223	1				
DM	0.393**	0.113	0.005	0.154	0.186	-0.113	-0.031	-0.088	1			
SC	0.297	0.309*	0.049	-0.053	0.004	0.207	0.073	-0.155	0.429	1		
1000-SW	-0.326	0.078	0.118	0.035	0.000	0.102	0.162	0.240	0.120	0.185	1	
Yield/plant	-0.012	0.055	0.000	0.106	0.280	0.410**	0.155	0.155	-0.108	0.140	0.462**	1

**Correlation is significant at the 0.01 level. *Correlation is significant at the 0.05 level. (50% F = 50% flowering, PH = Plant height, IL = Inter node length, P.B/Plant = Primary branch per plant, S.B/Plant = Secondary branch per plant, CP = Capsules per plant, CL = Capsule length, CD = Capsule diameter, DM = Days to maturity, SP = Seeds per Capsule and 1000-SW = 1000 seed weight.

observed by Arriel *et al.* (2007) and Pham *et al.* (2011). The dissimilarity distance matrix based on morphological data showed that the highest distance value was estimated between the genotypes RT 351 and Prachi. RT 351(white seeded) and Prachi (Black seeded) belong to two diverse groups (from II and V) in morphological cluster analysis but in case of molecular analysis it belongs to same cluster but in different sub cluster. Likewise, another pair could be EC-90 or EC 103 (both are black seeded) and Savitri (light brown seeded) genotypes occupying different cluster (I and II) with moderate genetic divergence value. Selection of parents based on parents belonging to highly diverse clusters need not necessarily produce top heterotic group (Arunachalam *et al.* 1984). On the contrary, moderate to genetic diversity group is sometimes more preferred to get better heterotic expression.

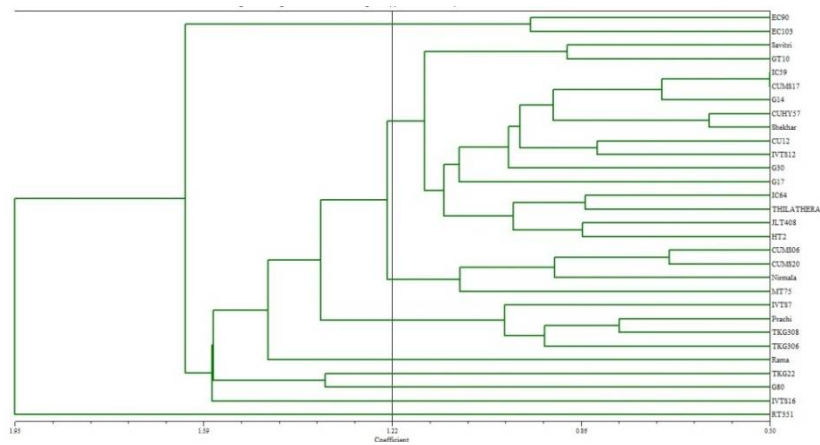


Fig. 1. Dendrogram showing the cluster pattern of 30 sesame genotypes based on quantitative characters.

Recently, the molecular markers microsatellites or SSRs are the most widely used for many purposes such as diversity, genome mapping and varietal identification etc. Though a large number of molecular markers have been employed in sesame genetic diversity studies, the majority of them were not based on the sesame genome sequence information (Ercan *et al.* 2004). In present study initially genotyping was carried out with 32 microsatellites but finally only 21 polymorphic markers were selected for 30 genotypes. These markers were found to provide polymorphic band among the population, revealing the variation present in the population. In SSR analysis a total of 93 alleles were observed which suggesting a high range of polymorphism present in the population (Table 4). The number of alleles per microsatellite locus varied from 3 (SSR 11, SSR 20, SSR 24) to 6 (SSR 12 and SSR 13) with an average of 4.42 alleles. The overall size of the amplified product varied from 80.69 bp (SSR 12) to 305.8 bp (SSR 30) for this experiment. This wide range reflects a large difference in the number of repeats between different alleles. The level of polymorphism was evaluated by calculating PIC value of each of the 21 primers selected for the study. The PIC values indicate the highly informative nature of these microsatellites. Estimated PIC value ranged from 0.07 to 0.87 and SSR 24 showed to be highest at 0.87. The results are in conformity with the findings of Cho *et al.* (2011). The dendrogram was constructed using the software NTSYS Pc (Ver. 2.20) based on 21 SSR markers in 30 sesame genotypes. All 30 genotypes were grouped into five main clusters (Fig. 2). Cluster I comprised of maximum number of genotypes i.e., 23 and it consisted of maximum number of genotypes developed from states of Rajasthan, Gujarat, Haryana, Madhya Pradesh, Uttar Pradesh,

Maharashtra, West Bengal and Orissa and one exotic collection. Cluster I contained five sub cluster, namely sub cluster A (contains 3 genotypes), sub cluster B (13 genotypes), sub cluster C (1 genotype), sub cluster D (5 genotypes), sub cluster E (1 genotype), 3 genotypes were present in cluster II, cluster III contained 2 genotypes, on the contrary cluster IV and cluster V contained only 1 genotype. Hence, it is clear from the clustering pattern that the genotypes belonging to different geographical origin were grouped together in the same cluster specially looking into cluster I. Genetic diversity of several varieties from the same zone may be distributed in different

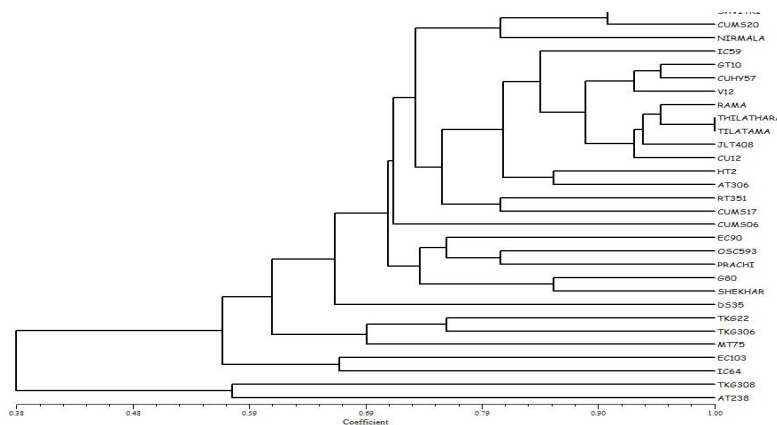


Fig. 2. Dendrogram showing the cluster pattern of 30 sesame genotypes based on SSR markers.

Table 4. Characteristic of SSR markers used in the genetic assessment of *S. indicum* L.

Sl. No.	Primer name	Range	No. of allele (A)	PIC value	Genetic diversity (H_e)	Effective allele (A_{ep})
1	CUSSR1	166.65-212.87	5	0.19	0.767	4.291
2	CUSSR2	200-268.69	5	0.31	0.866	7.462
3	CUSSR3	164.34-241.4	5	0.19	0.719	3.558
4	CUSSR5	89.63-191.94	4	0.13	0.759	4.149
5	CUSSR6	85.34-178.26	5	0.25	0.892	9.259
6	CUSSR7	100-205	5	0.46	0.851	2.386
7	CUSSR8	95.75-205.66	5	0.46	0.886	8.771
8	CUSSR10	165.86-211.47	5	0.36	0.845	6.451
9	CUSSR11	131.3-169.1	3	0.68	0.826	5.74
10	CUSSR12	80.69-136.43	6	0.36	0.781	4.566
11	CUSSR13	198.82-226.39	6	0.07	0.795	4.878
12	CUSSR14	178-229.1	5	0.36	0.822	5.617
13	CUSSR16	210.7-244.7	4	0.46	0.812	5.319
14	CUSSR17	157.36-186.12	4	0.13	0.582	2.392
15	CUSSR18	158.8194.3	5	0.36	0.856	6.944
16	CUSSR19	182.63-262.7	4	0.72	0.932	14.705
17	CUSSR20	160.45-193.7	3	0.41	0.802	5.05
18	CUSSR23	181.14-228.9	4	0.78	0.936	15.625
19	CUSSR24	162.04-182.1	3	0.87	0.936	15.625
20	CUSSR29	145-205.7	4	0.60	0.852	6.756
21	CUSSR30	211-305.8	4	0.78	0.856	6.944

PIC = Polymorphism information content.

clusters and similar findings were earlier reported by Pandey *et al.* (2015) in sesame. In the present study a high level of polymorphism was observed at the DNA level by SSR markers. The dissimilarity distance matrix based on molecular data showed that the similarity coefficients ranged from 0.01 to 0.95 with an average of 0.48 and the highest distance value was estimated between the genotypes Tilottama and CUMS-20 (Cluster 1 B and cluster 1A), Savitri and EC 90 (Cluster 1A and cluster 1 D). The genotypes EC 90 and Savitri through morphologically were distinctly divergent but at the molecular level they belonged to different sub cluster which delineated some divergence at the molecular level were also found between genotypes RT 351 and Prachi. So, crossing between these two parents would like to produce desirable segregants. The genetic dissimilarity estimates for 30 sesame genotypes were employed to generate a two-dimensional distribution plot by using PCA analysis with NTSYS Pc. 2.20. A data matrix plot based on 21 SSR primer descriptors was subjected to PCA for estimating genetic differentiation among 30 genotypes. In the present study, the two-dimensional distribution plot in factorial analysis revealed that the 30 genotypes were classified into five clusters. The plot showed that the genotypes, EC 103 and IC 64 were distinctly different from others. Cluster I comprised of maximum number of genotypes i.e. 21, cluster III contains 3 genotypes and rest of clusters *viz.* cluster II, cluster IV and cluster V comprised of only 2 genotypes, respectively. Most of the genotypes were scattered between these two varieties in the two dimensions matrix (Fig. 3). Principle components analysis and distance matrix analysis showed almost similar trend, but some variety did not corroborate with each other in cluster composition. Crossing between two distinctly related parents having maximum genetic distance or belonging to highly divergent group

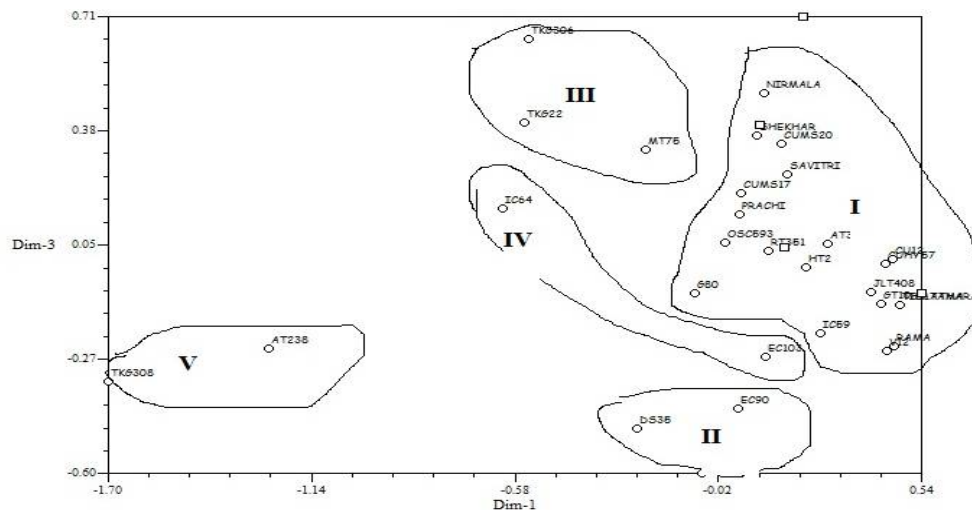


Fig. 3. 2D representation of PCA analysis in 30 sesame genotypes based on SSR marker data.

would like to generate transgressive segregant in segregating generation (Dasgupta and Das 1987). The dendrogram clearly indicated that many of the sesame genotypes collected from the same geographic locations did not cluster together. Distribution of genotypes did not reveal the relationship between geographical origin and grouping of genotypes. This result matched with Saha *et al.* (2012) and Iqbal *et al.* (2016). The usual assumption that selecting genotypes of different geographical origin will maximize the chance of producing segregation which may leads to diversity does not follow in sesame.

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