

Evolutionary Pattern on Genetic Resources and Diversity in Indian Land Races of Opium Poppy (*Papaver somniferum* L.)

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Abstract

The present study describes about genetic resources and diversity of the 55 opium poppy accessions/land races of Indian origin by means of D2-statistical analysis based on Mahalanobis; generalised distances. These lines belong to the different eco-geographical origin and are being maintained at CSIR-CIMAP, Lucknow. Fifty five poppy land races/genetic stocks were evaluated in the experimental field for two consecutive years (2015-16 and 2016-17). The collections were grown in randomized block design (RBD) with three replications. The Pooled mean data of two years were statistically analyzed using Institute's statistical Software 4.0 version which is available in the Genetics and Plant Breeding Division of the institute using Mahalanobis's generalized distance D2-statistics and canonical analysis. The clustering was done on the basis of Tocher's methods. The analysis of variance revealed higher significant differences for all the characters demonstrating considerable variation among them. The genotypes were grouped in seven diverse clusters (Cluster I - Cluster VII). Cluster I consist of 36 genotypes is the largest group followed by Cluster II, consisting 11 genotypes and cluster III has 3 genotypes while rest 3 clusters have only a single unique individual. The maximum intra-cluster distance was observed in cluster III (185.5) and minimum for Cluster IV (116.94). Inter-cluster D2 values range from 198.46 to 1285.68. The minimum was between Cluster I and IV (198.46) and maximum between Cluster IV and VII (1285.68). The trait codeine content (32.866%) was the highest contributor followed by papaverine content (105.41%) towards the divergence and days to maturity was the lowest contributor. Accessions/genotypes number 2 (A 2), 25 (A 32), 53 (A 74) may be explained in hybridization programme for opium poppy crop improvement.

Keywords: Cluster; D2-Statistics; Genetic Diversity; Opium Poppy; Variability

Introduction

The opium poppy, *Papaver somniferum*, is one of mankind's oldest medicinal plants. Opium poppy (*Papaver somniferum* L.) is primarily grown for opium latex and its valuable alkaloids viz. morphine, codeine, thebaine, narcotine and papaverine etc, which are used to prepare life saving drugs by pharmaceutical industries. The opium latex is obtained from green but fully mature capsules of the plant. It is a self-pollinated crop with varied degree of out-crossing (2 - 70%) depending upon the varieties, wind velocity, visits of bees and insects and climatic conditions [1,2].

The international consumption and demand for opium latex and its specific alkaloids thebaine and codeine, have increased multi-fold. In current days scientist of opium poppy are shifting on the development of CPS (concentrated poppy straw) varieties. This is essential to stop illegal practice and drug abuse arises from opium latex. To meet the demand, the plant breeders are actively engaged in the development of high yielding varieties, rich in specific alkaloids in poppy straw through various approaches of genetic manipulation. India is the largest legal producer and exporter of opium latex and its derivatives to the global market. Therefore,

to ful-fill this increasing demand of CPS varieties further genetic improvement and development of high yielding straw varieties rich in specific alkaloids is need of hour. Hybridization between selected diverse parental lines followed by selection is two most critical steps to achieve the desired genetic improvement [3-5]. The extent of genetic diversity between parents has been proposed as a predictor of F1 performance and magnitude of heterosis [6].

Poppy seeds are characterised by very good dietetic properties. The main component is oil (46 to 50% of dry matter), in which linoleic acid predominates (60 - 70%). Therefore, the dietetic properties of poppy oil are similar to those of sunflower oil. The seeds contain, among others, tocopherols, pantothenic acid, niacin, thiamine and a very high content of calcium (1400 mg/100g).

The present investigation was carried out to study, the degree of genetic divergence and variability present in the 55 germplasm/ Indian landraces of *Opium poppy* based on thirteen traits for further exploitation/utilization of identified diverse genotype(s) in heterosis breeding program for CPS opium cultivation/ crop improvement.

Material and Methods

The 55 opium poppy accessions included different breeding lines and landraces which have been maintained at the CSIR- Central Institute of Medicinal and Aromatic Plants, (CIMAP), Lucknow U.P. (India) for several years as inbred (Table 1). The opium poppy lines were grown in two consecutive years in field trials (2015-2016 and 2016-2017). In the trial a randomised complete block design with three replications was used for evaluation.

S. No.	Code	Details	Origin/Places
1.	A1	D3	CIMAP, Lucknow U.P. (India)
2.	A2	CIMAP-Ajay	CIMAP, Lucknow U.P. (India)
3.	A3	Df	CIMAP, Lucknow U.P. (India)
4.	A5	I 14	CIMAP, Lucknow U.P. (India)
5.	A6	N-3	CIMAP, Lucknow U.P. (India)
6.	A7	G 25	109/bulk Disease sucept. IARI, N. Delhi
7.	A8	G 41	Material 116 IARI, N. Delhi (India)
8.	A9	Thailand-1	Thailand
9.	A11	P 26	CIMAP, Lucknow U.P. (India)
10.	A13	Thai-4	Thailand

11.	A14	P 34	CIMAP, Lucknow U.P. (India)
12.	A15	3004A	CIMAP, Lucknow U.P. (India)
13.	A16	Inb 37	CIMAP, Lucknow U.P. (India)
14.	A17	Popu 40	CIMAP, Lucknow U.P. (India)
15.	A18	Pop 44	CIMAP, Lucknow U.P. (India)
16.	A20	IS 19	CIMAP, Lucknow U.P. (India)
17.	A21	IS 16	CIMAP, Lucknow U.P. (India)
18.	A22	IS 21	CIMAP, Lucknow U.P. (India)
19.	A23	IS 22	CIMAP, Lucknow U.P. (India)
20.	A24	IS 23	CIMAP, Lucknow U.P. (India)
21.	A25	IS 26	CIMAP, Lucknow U.P. (India)
22.	A26	Vivek	CIMAP, Lucknow U.P. (India)
23.	A30	IS 44	CIMAP, Lucknow U.P. (India)
24.	A31	IS 47	CIMAP, Lucknow U.P. (India)
25.	A32	SPS 20	CIMAP, Lucknow U.P. (India)
26.	A33	IS 22	CIMAP, Lucknow U.P. (India)
27.	A34	IS 23	CIMAP, Lucknow U.P. (India)
28.	A35	SPS 24	CIMAP, Lucknow U.P. (India)
29.	A36	IS 25	CIMAP, Lucknow U.P. (India)
30.	A37	IS 26	CIMAP, Lucknow U.P. (India)
31.	A38	IS 34	CIMAP, Lucknow U.P. (India)
32.	A39	IS 36	CIMAP, Lucknow U.P. (India)
33.	A40	IS 45	CIMAP, Lucknow U.P. (India)
34.	A41	Subhra	CIMAP, Lucknow U.P. (India)
35.	A42	GS 11	Ghazipur U.P. (India)
36.	A43	GS 18	Ghazipur U.P. (India)
37.	A47	Shweta	Ghazipur U.P. (India)
38.	A48	U N-1	CIMAP, Lucknow U.P. (India)
39.	A49	C 54	CIMAP, Lucknow U.P. (India)
40.	A50	HSY 8	CIMAP, Lucknow U.P. (India)
41.	A51	HSY 26	CIMAP, Lucknow U.P. (India)
42.	A53	Thai 5	Thailand
43.	A54	UN 1	CIMAP, Lucknow U.P. (India)
44.	A55	UN 2 Prick	CIMAP, Lucknow U.P. (India)
45.	A57	TS 3	CIMAP, Lucknow U.P. (India)
46.	A58	GS 23	Safada Bad U.P. (India)
47.	A59	GS 25	Ghazipur U.P. (India)
48.	A60	UN 7	Violet U.P. (India)
49.	A62	GS 27	Ghazipur U.P. (India)
50.	A65	Un 12	CIMAP, Lucknow (India)
51.	A66	IS 14	CIMAP, Lucknow (India)

52.	A73	Shyama	CIMAP, Lucknow (India)
53.	A74	Sanchita	CIMAP, Lucknow (India)
54.	A75	Sampada	CIMAP, Lucknow (India)
55.	A79	HSY 23	CIMAP, Lucknow (India)

Table 1: Genetic stocks/ accessions, their codes and origin of the opium poppy.

Experimental site

The present experiment was conducted at the experimental field of Genetics and Plant Breeding, CSIR-CIMAP, Lucknow, U.P. (India) located at 26.5° N latitude and 80.50° E longitude and 120m above sea level.

2016-17). The collections were grown in randomized block design (RBD) with three replications at the research farm at CSIR-Central Institute of Medicinal and Aromatic Plants (CIMAP), Lucknow, India. Plants were planted in rows 4m long and 50 cm apart. The plants received normal intercultural operations, irrigation, and fertilizer applications (120 kg N, 80 kg P₂O₅, and 60 kg K₂O.ha⁻¹). Morpho-metric data were recorded on five competitive randomly selected plants in each line for following thirteen traits- days to 50% flowering, plant height (cm), capsules/plant, capsule size, seed yield(g)/plant, capsule husk yield (g)/plant, alkaloid content (%) in poppy straw includes five major alkaloid i.e. morphine, codeine, thebaine, papaverine and narcotine (Figure 1-3).



Figure: Experimental site/ location of the study.

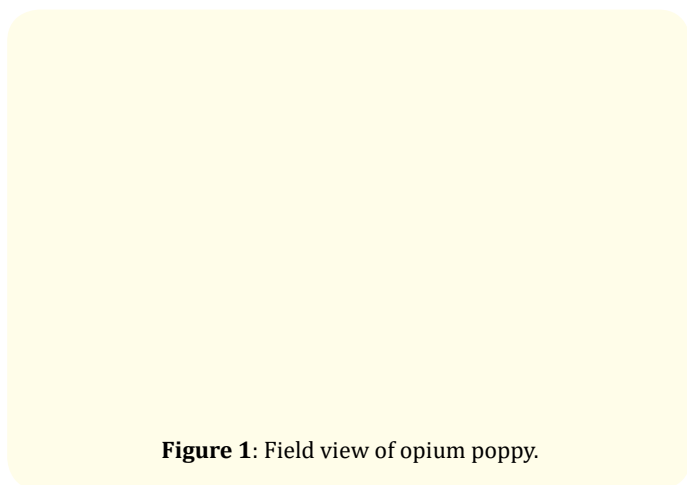


Figure 1: Field view of opium poppy.

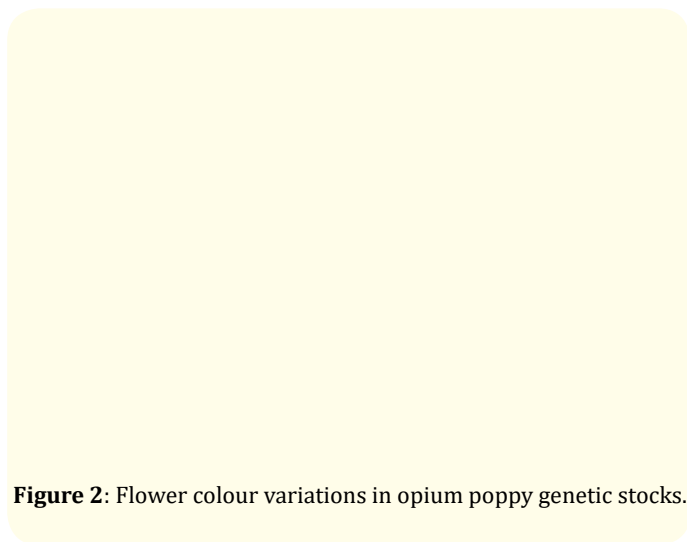


Figure 2: Flower colour variations in opium poppy genetic stocks.

Experimental Design

All analysed 55 poppy landraces/genetic stocks were cultivated in the experimental field for two consecutive years (2015-16 and

Figure 3: Leaf margin variations in opium poppy genetic stocks.

Statistical analysis

The Pooled mean data of two years were statistically analyzed using the CSIR-CIMAP, Lucknow developed Statistical Software 4.0 version which is available in the Genetics and Plant Breeding Division of the institute based on [7,8]. The data on 13 morpho-metric traits were analyzed using Mahanopolis’s generalized distance [9] D²-statistics and canonical analysis. The clustering was done on the basis of Tocher’s methods [10].

Results and Discussion

The ANOVA showed significant differences among the genotypes for all the thirteen characters studied, which indicated the presence of high genetic variability among the present set of materials (Table 2). Cluster I was the largest group that comprised 36 genotypes followed by Cluster II, consisted of 11 genotypes and clusters III had 3 genotypes and Cluster 4 had 2 genotype. Rest 3 clusters, Cluster V, VI and VII, have single genotype in each (Table 3). The maximum intra-cluster distance was observed in cluster III (185.5) and minimum for Cluster IV (116.94). Inter-cluster D2 values range from 198.46 to 1285.68. The minimum distance was between Cluster I and IV (198.46) and maximum distance between Cluster IV and VII = 1285.68 (Table 4).

Based on the cluster means the important cluster for high morphine content was Cluster V that possesses highest morphine content (0.352%) and codeine content (0.297) low or negligible papaverine along with low seed (5.112g) in poppy straw. Cluster VI had the highest mean for plant height (106.3 cm), capsule index (0.998) and negligible papaverine. Cluster VI had higher mean for days to flowering (106.1), with lowest plant height (99.23 cm) respectively.

Sl. No.	Traits	Replication (MS) df = 2	Treatment (MS) df = 54	Error (MS) df = 108
1	Days to 50% flowering	2.3125	13.4120**	1.1574
2	Plant height (cm)	1.6875	28.3056**	6.1412
3	Pedicle length (cm)	1.81633	27.67206**	2.777131
4	Days to maturity (days)	0.00808	19.76821**	0.38737
5	Number of capsule/plant	0.14209	1.90079**	0.09682
6	Capsule index	0.00015	0.01771**	0.00247
7	Seed yield (g)/plant	0.33401	3.27562*	1.61781
8	Poppy husk yield (g)/plant	0.65184	1.26296*	0.51930
9	Morphine content (%)	0.0002	0.0225**	0.0003
10	Codeine content (%)	0.0024	0.0120**	0.0007
11	Thebaine content (%)	0.00005	0.0003**	0.00000648
12	Papaverine content (%)	0.0000005	0.0003**	0.0000009
13	Narcotine content (%)	0.0000005	0.0004**	0.000001

Table 2: ANOVA for thirteen characters of opium poppy accessions. **= p < 0.01, respectively.

S. No.	Cluster	Number of accessions	Accessions serial number
1	I st	36	3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 19, 23, 26, 28, 30, 31, 32, 33, 37, 38, 39, 40, 42, 43, 44, 46, 47, 51, 52, 54, 55
2	II nd	11	18, 20, 24, 27, 29, 34, 36, 41, 45, 48, 49
3	III rd	3	21, 22, 50
4	IV th	2	1, 35
5	V th	1	2
6	VI th	1	25
7	VII th	1	53
Total	7	55	55

Table 3: Distribution of 55 accessions in different cluster of opium poppy.

Cluster	I	II	III	IV	V	VI	VII	\bar{D}^2
I	146.32 (12.0962)	210.78 (14.5182)	395.51 (19.8874)	198.46 (14.0875)	844.98 (29.0685)	611.44 (24.7273)	951.59 (30.8478)	479.86
II		164.634 (12.8309)	226.34 (15.0446)	199.99 (14.1417)	601.43 (24.5244)	508.86 (22.5579)	680.44 (26.0852)	370.35
III			185.555 (13.6218)	301.56 (17.3654)	458.18 (21.4051)	675.49 (25.9901)	451.44 (21.2471)	384.86
IV				116.943 (10.8140)	735.25 (27.1154)	754.88 (27.4750)	532.19 (23.0692)	405.62
V					0 0	604.15 (24.5794)	802.05 (28.3204)	578.00
VI						0 0	1285.68 (35.8563)	634.35
VII							0 0	4703.39

Table 4: Intra and Inter cluster divergence (\bar{D}^2) among seven cluster of opium poppy.

\bar{D}^2 = Mean of D2

Cluster VI had highest mean for, seed yield (10.344g). Cluster V has high mean values for capsule husk yield (5.35g), codeine (0.297%), respectively. Cluster VII includes genotypes having high peduncle length and high papaverine content while these have low mean values for days to flowering (Table 5). It is reported that genotypes within the cluster with a high degree of divergence would produce more desirable breeding materials for achieving maximum genetic gain [11-15]. From the obtained results it could be

concluded that accession with high seed yield in cluster VI, accessions with low codeine, thebaine content from cluster III could be selected as parents for the hybridization program to fulfil the objective of poppy with high seed yield and low amount of alkaloid in the development of CPS varieties. Accessions among the cluster separated by high D²-values could be further used in the hybridization program for obtaining a wide range of variations among the segregates.

Cluster	DF	PH	PL	DM	NC	CI	SY	CHY	M	C	T	P	N
I	101.9	102.7	22.19	122.09	2.163	0.837	8.2011	4.5004	0.038	0.039	0.012	0.0104	0.0162
II	103.8	103.2	22.04	124.1	2.616	0.827	9.2156	4.0167	0.143	0.011	0.016	0.0124	0.012
III	102.8	102.6	20.76	126.4	2.075	0.906	7.7634	4.8959	0.257	0.002	0.003	0.017	0.018
IV	100.9	101.8	23.35	120.5	1.5556	0.851	8.02222	4.66834	0.115	0.015	0.005	0.025	0.003
V	101.5	99.66	18.84	128.9	1.2222	0.833	5.1123	5.352	0.352	0.297	0.037	0.004	0.007
VI	106.1	99.23	18.76	128.3	1.5556	0.752	10.344	4.41334	0.135	0.065	0.056	0.014	0.038
VII	100.7	106.3	24.02	125.7	2.778	0.998	7.900	4.90334	0.311	0.0046	0.006	0.062	0.007

Table 5: Cluster mean of the thirteen economic traits in opium poppy accessions.

Where; DF: Days to 50% flowering; PH: Plant Height (cm); PL: Peduncle Length (cm); DM: Days to Maturity; NC: Number of Capsule/Plant; CI: Capsule Index; SY: Seed Yield/Plant (g); CHY: Capsule Husk Yield/Plant; M: Morphine Content (%); C: Codeine Content (%); T: Thebaine Content (%); P: Papaverine Content (%); N: Narcotine Content (%).

Codeine (32.866%) was the highest contributor followed by papaverine (10.541%) towards the divergence and days to maturity (0.277%) were the lowest contributor. The grouping obtained through D²-statistics based on two dimensional Z₁ and Z₂ chart, the contribution of characters towards divergence of genotypes revealed that in vector (Z₁) contribution toward genetic divergence in the primary axis of differentiation were due

to codeine, morphine (68.80%) found in days to maturity (30.445%) and lowest was narcotine content (-9.432%). In vector II (Z₂) which was the secondary axis of differentiation, days to 50% flowering (61.83%), thebaine (46.83%) and narcopine (41.74%) were important (Table 6 and Figure 4).

Parameters	DF	PH	PL	DM	NC	CI	SY	CHY	M	C	T	P	N
Mean (\bar{X})	102.32	102.38	22.184	123.38	2.123	0.836	8.351	4.524	0.0833	0.036	0.01166	0.0129	0.0160
SEM	0.62113	1.4307	0.9611	0.3557	0.1796	0.0287	0.7343	0.4160	0.0097	0.0151	0.00146	0.0017	0.0018
CD _(0.05) %	1.7392	4.0063	2.6913	0.9960	0.5030	0.08036	2.0562	1.1650	0.0271	0.0424	0.00410	0.0049	0.0052
CD _(0.01) %	2.3102	5.3215	3.5749	1.3230	0.6681	0.1067	2.7312	1.5474	0.0360	0.0563	0.00545	0.0065	0.0069
CC (%)	0.477	1.372	3.407	0.227	6.654	2.701	6.915	7.231	9.155	32.866	9.414	10.541	9.040
Ranks	XII	XI	IX	XIII	VIII	X	VII	VI	IV	I	III	II	V
Z ₁ Vector	0.05462	0.0392	-0.044	0.4425	-0.103	0.0223	-0.0639	0.0678	1.000	0.0675	0.05761	0.0509	-0.137
CTPA (%)	3.7580	2.699	-3.041	30.445	-7.148	1.53430	-4.3971	4.6648	68.8027	4.6441	3.9018	3.5068	-9.432
CCPAR	V	VIII	X	II	XII	IX	XI	III	I	IV	VI	VII	XIII
Z ₂ Vector	1.000	-0.1779	-0.130	0.6306	-0.369	-0.1721	-0.2113	0.0841	-0.0128	0.2099	0.7561	-0.664	0.6751
CTSA (%)	61.8272	-10.626	-8.093	38.988	-22.82	-10.640	-13.064	5.2046	-0.7957	12.977	46.7475	-41.07	41.739
CCSAR	I	IX	VIII	IV	XII	X	XI	VI	VI	V	II	XIII	III
Range (\bar{X})	98.77 - 106.21	96.06 - 108.28	17.45 - 28.2	118.89 - 128.8	1 - 3.777	0.7244 - 1.0166	5.1122 - 10.344	3.28 - 6.61	0.006 - 0.309	0.004 - 0.297	0.003 - 0.0307	0.003 - 0.062	0.003 - 0.054

Table 6: Range; Character contributions (%); rank and other allied genetic parameters of the thirteen economic traits in *Papaver somniferum* accessions.

Where: DF: Days to 50% flowering; PH: Plant Height (cm); PL: Peduncle Length (cm); DM: Days to Maturity; NC: Number of Capsule/Plant; CI: Capsule Index; SY: Seed Yield/Plant (g); CHY: Capsule Husk Yield/Plant; M: Morphine Content (%); C: Codeine Content (%); T: Thebaine Content (%); P: Papaverine Content (%); N: Narcotine Content (%); CC %: Character Contributions (%); CTPA (%): Percent Contribution Towards Primary Axis; CCPAR: Character Contribution at Primary Axis Rank; CTSA (%): Percent Contribution Towards Secondary Axis; CCSAR: Character Contribution at Secondary Axis Rank.

Conclusions

Genetic divergence among 55 genetic stock of opium poppy to represent a wide range of variation with respect to yield has been estimated using D2-statistics. Selection of promising accessions based on genetic divergence along with better seed yield. Selected five promising accessions on the basis of genetic divergence with better seed yield A-43 (10.411 gm), A-32 (10.344 gm), A-40 (10.044 gm), A-24 (9.767 gm), and A-18 (9.8 gm). For Capsule husk yield accession A-40 (6.61 gm), A-39 (6.23 gm), A-59 (5.483 gm), A-60 (5.38 gm) and A-11 (5.15 gm) were found promising. On the basis of observation recorded in divergent clusters of poppy it can be concluded that hybridization between diverse parents among different clusters may be useful and help in coping with limitation pertaining with the improvement of poppy.

Figure 4: Spatial distribution of 55 accessions in opium poppy.

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