Alkaloid Diversity in Relation to Breeding for Specific Alkaloids in Opium Poppy (*Papaver somniferum* L.)

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Abstract: *Papaver somniferum* is a chief source of diverse physiologically active alkaloids, required by the pharmaceutical industry. The present study describes the diversity of the alkaloid spectrum of 122 opium poppy accessions of Indian origin by means of a cluster analysis based on Mahalanobis generalised distances. The accessions could be grouped into 11 clusters according to their relationship between the contents of morphine, codeine, thebaine, narcotine and papaverine in raw opium. The diversity of the alkaloid spectrum of 11 clusters reflected the very low correlations between the contents of the individual alkaloids across the 122 entries, found earlier. The clusters represented almost all possible combinations of the high content of an alkaloid with high or low content of another alkaloid. Although on average the morphine content exceeds the sum of the other four alkaloids, in one cluster the narcotine content (15.3%) was even higher than that of morphine (14.6%) and the content of the remaining alkaloids was also extremely high. The variation range among the clusters was for papaverine between 0.14% to 5.3%, while for morphine between 12.4% to 18.0%. The results indicate a large space for the breeding of opium poppy for individual alkaloids or particular combinations of alkaloids, as required by pharmaceutical industries.

Keywords: alkaloids; clustering; multivariate; *Papaver somniferum*

Opium poppy (*Papaver somniferum* L.) is the main source of raw opium, which contains valuable alkaloids utilised by pharmaceutical industries, i.e. mainly morphine, codeine, thebaine, narcotine and papaverine. India is one of the largest producers of opium which meets national and international demands. Global trends show the growing utilisation of opium alkaloids and derivatives. The world demand for morphine, the main alkaloid of opium, and for codeine was 420 t and 350 t, respectively, in 2008. The domestic and international demand for thebaine-based drugs increased from 140 t in 2008 to 160 t in 2009. Thebaine is used to manufacture semi-synthetic morphine analogues i.e. oxycodone, oxymorphine, buprenorphine etc. The increasing demand for opium alkaloids can be met by the development of high opium yielding varieties able to produce the specific alkaloids. Successful breeding, however, requires sufficient genetic diversity to start from. Several studies have been done to determine the genetic diversity in opium poppy based on morphological traits (Lal *et al.* 1996; Bhandari *et al.* 1997; Saini & Kaicker 1997; Singh *et al.* 1998, 2003, 2004; Tiwari *et al.* 2001), but few attempts have been made to classify the diverse germplasm lines on the basis of alkaloids. Shukla *et al.* (2006) investigated the alkaloid profile of 98 accessions of opium poppy and found large variability among the accessions in all five alkaloids. Yadav *et al.* (2006) investigated the genetic parameters and correlations

between the five alkaloids in a collection of 122 opium poppy accessions. They found large genetic variation in the content of all five alkaloids (Table 1), but low genetic correlations between the five alkaloids (the strongest was $r = 0.37$), indicating almost independent inheritance of the alkaloids and thus a high potential for genetic improvement by breeding. The present study tries to group these 122 accessions into clusters, using the analytical data obtained by Yadav *et al.* (2006), in order to facilitate the selection of suitable parents for breeding poppy varieties with a desired alkaloid spectrum.

MATERIAL AND METHODS

The 122 opium poppy accessions included different breeding lines and landraces which have been maintained at the National Botanical Research Institute (NBRI), Lucknow, India for several years as pure lines. They are grouped by origin in Table 2. The opium poppy lines were grown in a two-year field trial, performed by Yadav *et al.* (2006) in the crop years 2002-03 and 2003-04 at the NBRI experimental site at Lucknow. The trial design was a randomised complete block with three replications. A detailed description of the experimental design, description of the environmental conditions of the site, cultural practices and the details of the analytical procedures for the determination of alkaloids (HPLC) were given by Yadav *et al.* (2006).

To show the similarities between the alkaloid spectra of the entries the analytical data on the five alkaloids, obtained by Yadav *et al*. (2006) for 122 poppy accessions, were used to perform a hierarchical cluster analysis based on the average linkage method.

Table 1. Variations in the content of five alkaloids in the opium of 122 accessions of opium poppy (*Papaver somniferum* L.); according to Yadav *et al.* (2006), modified

Alkaloid (%)	Minimum	Mean	Maximum	SD	*CV* (%)
Morphine	9.20	15.41	20.86	2.58	17.00
Codeine	1.57	3.21	6.76	0.88	28.66
Thebaine	0.61	2.05	8.36	1.15	57.01
Narcotine	2.27	8.18	17.92	2.68	32.95
Papaverine	0.00	1.25	6.04	1.56	125.97

SD = standard deviation of the whole population, *CV* = coefficient of variation

Table 2. The origin of accessions used for an assessment of genetic diversity in the content of major alkaloids of opium poppy (*Papaver somniferum* L.)

	Accessions	Location/origin
1	BR 001, BR 002, BR 003, BR 004, BR 006, BR 007, BR 008, BR 009, BR 010A, BR 311, BR 329, BR 010B, BR 321, BR 331, BR 314, BR 315, BR 316, BR 334, BR 312, BR 313	landraces from different districts of Uttar Pradesh (U.P.) state, India
2	BR 275, BR 271, BR 286, BR285, BR 284, BR 283, BR 282, BR 281, BR 280, BR 279, BR 278, BR 277, BR 276, BR 272, BR 273, BR 274, BR 320, BR 304, BR 301, BR 303, BR 300, BR 302, BR 317, BR 319, BR 318	collections, New Delhi, India
3	BR 292, BR 294, BR 293, BR 295, BR 296, BR 297, BR 298, BR 299, BR 289, BR 288, BR 287, BR 291, BR 247, BR 248, BR 249, BR 250, BR 251, BR 254, BR 255, BR 256, BR 257, BR 258, BR 259, BR 263, BR 264, BR 265.	collections from different districts of Rajasthan state, India
4	BR 306, BR 310, BR 305, BR 309, BR 307, BR 308, BR 252, BR 253, BR 260, BR 261, BR 262, BR 266, BR 267, BR 268, BR 269, BR 270.	collections from different districts of Madhya Pradesh (M.P.) state, India
5	BR 046, BR 047A, BR 047B, BR 048, BR 049, BR 050, BR 051, BR 052, BR 053, BR 054, BR 055, BR 056A, BR 056B, BR 057, BR 058, BR 060, BR, 061, BR 062, BR 063, BR 064, BR 065, BR 066, BR 067, BR 068.	developed through interspecific cross at NBRI , Lucknow, India
6	BR 005, BR 322, BR 323, BR 324, BR 325, BR 222, BR 220, BR 326, BR 327, BR 328.	improved breeding lines, NBRI, Lucknow, India

Mahalanobis generalised distances (D^2) according to Rao (1952) were calculated within and between the clusters, as a measure of similarity between the alkaloid spectra of the accessions. A dendrogram, based on the linkage index between the entries, was produced to visualise the clusters and the similarities of the alkaloid spectra. All calculations were performed by means of the WINDOWSTAT software package of Indostat services, Hyderabad.

RESULTS AND DISCUSSION

The results of the cluster analysis are summarised in Figure 1.The clustering produced 11 clusters depending upon the similarity of the alkaloid spectrum of 122 accessions. The accessions belonging to the individual clusters are listed in Table 3, together with the geographical origin of each accession given in parentheses. The cluster size varied from 4 to 25. All clusters contained accessions of different origin, except the small cluster VII, with all accessions originating from New Delhi. Also, accessions of common geographical origin were distributed over several clusters. This indicates the limited role of geographical distribution in the genetic diversity of opium poppy of Indian origin, as was demonstrated also by Singh (1991), Singh *et al.* (2003, 2004). Because of the different cluster size, the large clusters included most of the accessions.

The inter-cluster and the mean intra-cluster Mahalanobis distances are summarised in Table 4. The mean content of the five alkaloids of each cluster is given in Table 5. The mean intra-cluster distance (the mean of all distances within a cluster) is a measure of variability within a cluster. The values of inter-cluster distances were, as should be expected, greater than the mean distances within clusters. Among all clusters, the smallest cluster VII, containing four accessions from New Delhi, was unique in several respects. It had the highest intra-cluster variability but it was also the most distant from the

Table 3. Distribution of 122 accessions into 11 clusters based on major alkaloids of opium poppy (*Papaver somniferum* L.)

Cluster	No. of accessions	Accessions
I	25	BR001(U.P.), BR275 (ND), BR009(U.P.), BR271(ND), BR010A(U.P.), BR066(IS), BR277(ND), BR047A(IS), BR293(Raj), BR050(IS), BR010B(U.P.), BR321(U.P.), BR301(ND), BR319(ND), BR048(IS), BR052(IS), BR220(IV), BR313(U.P.), BR063(IS), BR064(IS), BR065(IS), BR067(IS), BR308(M.P.), BR312(U.P.), BR328(IV)
II	20	BR005(U.P.), BR272 (ND), BR318(ND), BR324(IV), BR273(ND), BR323(IV), BR329(U.P.), BR298(Raj), BR334(U.P.), BR056B(IS), BR258(Raj), BR007(U.P.), BR286(ND), BR305(M.P.), BR062(IS), BR299(Raj), BR322(IV), BR315(U.P.), BR053(IS), BR310(M.P)
III	4	BR295(Raj), BR304(ND), BR278(ND), BR316 (U.P)
IV	19	BR002(U.P.), BR061(IS), BR306(M.P), BR285(ND), BR006(U.P.), BR054(IS), BR056A(IS), BR004(U.P.), BR008(U.P.), BR325(IV), BR320(ND), BR003(U.P.), BR274(ND), BR292(Raj), BR311(U.P.), BR280(ND), BR302(ND), BR331(U.P.), BR300(ND)
V	13	BR247(Raj), BR263(Raj), BR248(Raj), BR264(Raj), BR260(M.P.), BR268(M.P.), BR254(Raj), BR252(M.P.), BR261(M.P.), BR327(IV), BR253(M.P.), BR262(M.P.), BR269(M.P.)
VI	6	BR249(Raj), BR259(Raj), BR250(Raj), BR265(Raj), BR267(Raj.), BR068(IS)
VII	4	BR283(ND), BR282(ND), BR281(ND), BR284(ND)
VIII	11	BR276(ND), BR049(IS), BR256(Raj), BR257(Raj), BR255(Raj), BR266(M.P.), BR270(M.P.), BR251(Raj), BR307(M.P.), BR291(Raj), BR047B(Raj)
IX	5	BR314(U.P), BR055(IS), BR222(IV), BR317(ND), BR051(IS)
X	9	BR294(Raj), BR057(IS), BR290(Raj), BR287(Raj), BR303(ND), BR046(IS), BR297(Raj), BR326(IV), BR288(Raj)
XI	6	BR296(Raj), BR289(Raj), BR279(ND), BR309(M.P.), BR060(IS), BR058(IS)

U.P. = landraces from Uttar Pradesh; M.P. = collections from Madhya Pradesh; Raj = collections from Rajasthan; ND = collections from New Delhi; IS = developed through interspecific cross

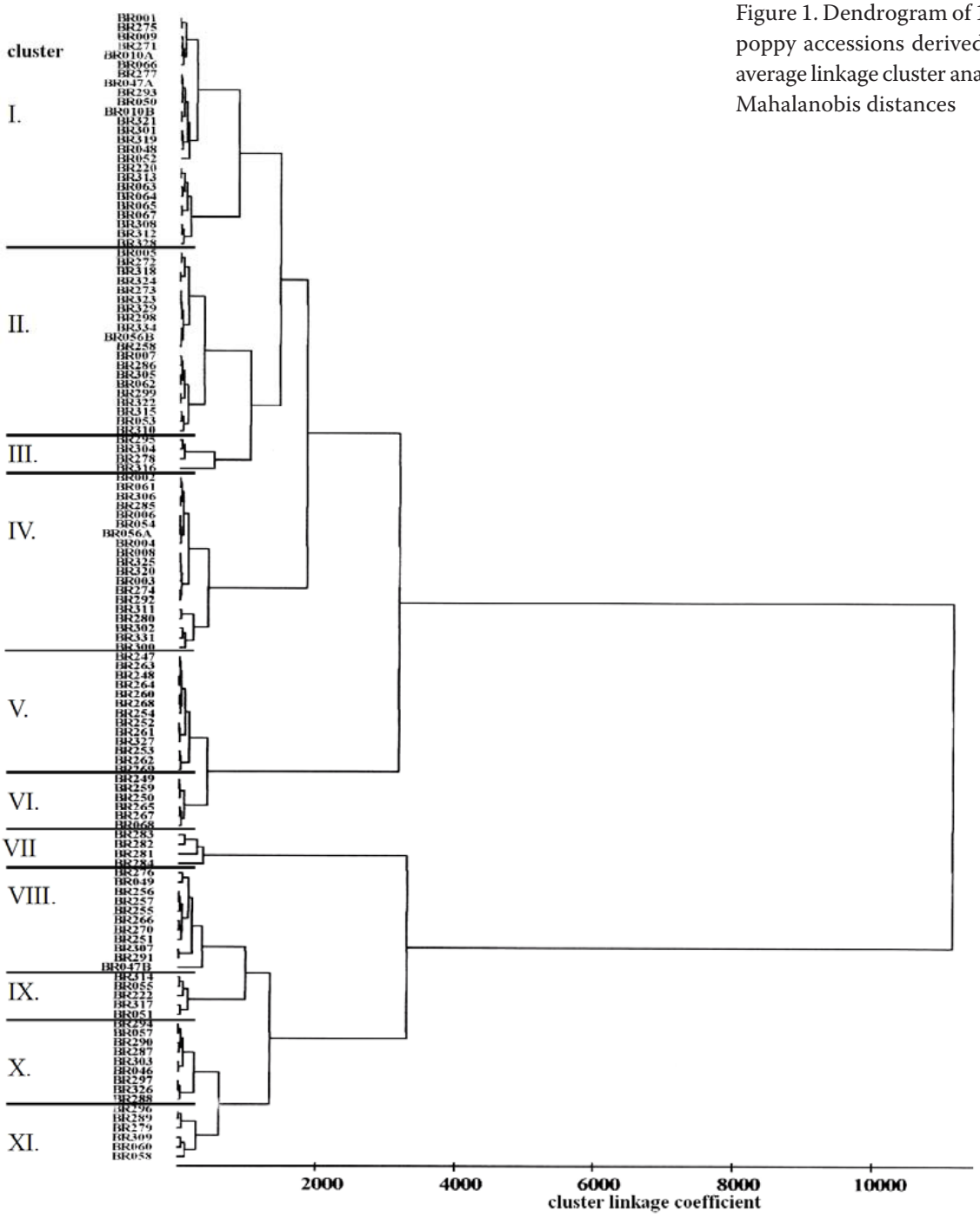

Figure 1. Dendrogram of 122 opium poppy accessions derived from the average linkage cluster analysis using Mahalanobis distances

other clusters. A look into Table 4 reveals that this cluster contains accessions with extraordinarily high content of codeine, thebaine, narcotine and papaverine, but only moderate content of morphine. This is in strong contrast with the remaining accessions, in which the average morphine content is higher than the sum of the four other alkaloids.

Since the heritabilities of the content of each alkaloid of 122 accessions are extremely high, as found by Yadav *et al.* (2006), the Mahalanobis distance is a good measure of genetic distance. The distance between clusters can therefore be effectively used to select genetically distant parents for hybridization programmes, aimed at obtaining high-yielding opium poppy lines with high content of particular alkaloids or combinations of alkaloids. It is common knowledge that more heterosis can be expected in hybrids of genetically more distant parents than in those of genetically close parents, as shown for example in hyacinth beans (Singh 1991). The genetic divergence

Table 4. Intra- (diagonal bold) and inter-cluster Mahalanobis distances for 122 accessions in opium poppy (*Papaver somniferum* L.)

Clusters	I	II	III	IV	V	VI	VII	VIII	IX	X	XI
I	163.67	244.36	583.21	318.99	394.13	372.94	2164.73	672.53	1328.31	401.12	884.42
II		91.69	502.01	208.83	261.96	178.19	2113.14	621.18	1359.32	289.94	783.54
III			404.58	793.37	629.20	591.96	2454.33	988.54	1677.30	714.01	1295.20
IV				121.83	537.76	482.42	1742.30	852.11	1434.28	363.09	681.04
V					63.69	155.39	2053.76	227.73	810.38	208.36	650.72
VI						60.96	2676.09	509.97	1375.97	370.48	1006.46
VII							485.06	1675.31	995.97	1310.15	719.19
VIII								180.25	419.58	269.96	503.58
IX									138.13	576.75	410.32
X										107.88	301.26
XI											210.38

seems to be an important tool for the selection of prospective parents for further utilization in any crop improvement programme (Singh *et al.* 2003, 2004). Mean performance of different clusters for major alkaloids revealed that the accessions with the lowest morphine and papaverine content were included in cluster I, however, this cluster had accessions with the second highest codeine content. On the other hand, cluster VI, which consisted of six accessions, five from Rajasthan and one from an interspecific cross, had the highest morphine and the lowest codeine, thebaine and narcotine content, as well as the second lowest papaverine content, accompanied by the lowest intra-cluster distance. Contrary to this, the accessions with the highest codeine, thebaine, narcotine and papaverine content and the third lowest morphine content were grouped in cluster VII, which had a maximum intra-cluster distance. The means of clusters VI and VII indicated that an increase in morphine was associated with a decrease in other alkaloids and thus they showed negative associations. The probable reason for this increase or decrease in the alkaloids can be understood by studying the biosynthetic pathway of opium alkaloids (Shukla *et al.* 2006). The biosynthetic pathway of major opium alkaloids suggests that morphine, codeine, thebaine and narcotine are synthesized from reticuline whereas papaverine stems from nor-reticuline (Cordell 1981; Kutchan 1998). Thebaine is formed from reticuline, which is converted to morphine following two pathways. The first pathway involves the route thebaine → codeinone → codeine → morphine and the other thebaine → oripavine → morphinone → morphine (Psenak 1998). The predominance of one pathway over the other may depend upon the relative activities of 3-0 methyl oxidase/6-0 methyl oxidase. Any genetic block which hinders the conversion of nor-reticuline to papaverine would not block

Table 5. Cluster means and standard errors of the means of the content of five alkaloids in raw opium (in %)

Clusters	Morphine	Codeine	Thebaine	Narcotine	Papaverine
I	12.42 ± 0.30	3.65 ± 0.22	2.04 ± 0.17	7.78 ± 0.37	0.14 ± 0.05
II	17.42 ± 0.46	3.05 ± 0.10	1.91 ± 0.12	8.13 ± 0.22	0.21 ± 0.04
III	16.38 ± 1.03	3.56 ± 0.43	4.96 ± 1.16	5.87 ± 0.97	0.15 ± 0.14
IV	16.23 ± 0.35	3.31 ± 0.17	1.80 ±1.3	11.14 ± 0.34	0.40 ± 0.15
V	16.91 ± 0.39	3.21 ± 0.12	1.38 ± 0.06	5.44 ± 0.23	1.48 ± 0.12
VI	18.02 ± 0.71	2.13 ± 0.21	1.03 ± 0.11	5.32 ± 0.35	0.19 ± 0.08
VII	14.57 ± 1.46	4.72 ± 0.76	5.02 ± 0.84	15.26 ± 1.08	5.29 ± 0.43
VIII	14.79 ± 0.59	2.90 ± 0.27	1.48 ± 0.29	5.61 ± 0.41	2.78 ± 0.17
IX	12.58 ± 0.58	2.93 ± 0.19	2.54 ± 0.20	7.59 ± 0.58	4.81 ± 0.34
X	15.92 ± 0.61	2.67 ± 0.20	2.08 ± 0.23	8.47 ± 0.19	2.06 ± 0.20
XI	15.44 ± 1.25	3.09 ± 0.25	2.47 ± 0.44	11.02 ± 0.44	3.54 ± 0.23
Mean ± SE	15.41 ± 0.55	3.21 ± 0.24	2.05 ± 0.24	8.18 ± 0.41	1.25 ± 0.14

alkaloid synthesis from reticuline so that the pathway of morphine synthesis can be normal. In the present investigation, the accessions with low morphine and high papaverine were located in one cluster (VII) and the accessions with low papaverine and high morphine in the other cluster (VI). So, the present study helped to identify some unique accessions of these clusters (VII, VI) having one or more genetic block(s) in the normal biosynthetic pathways. Further study of these accessions is undoubtedly needed to explain biosynthetic pathways for concerned regulatory signals/genetic block(s).

Cluster VII exhibited a maximum inter-cluster distance from cluster VI (2676.09). The accessions of this cluster can be used as a potential source for introgression of new genes into distantly related accessions for enhancing the specific alkaloids through a hybridization programme followed by selection.

It can be concluded from the present study that clusters VI, VII, II, III and IX showed a relatively greater potential for an increase in the content of certain alkaloids. A planned hybridization programme based on the intercrossing of promising accessions between different clusters may release high heterosis for specific alkaloids especially for thebaine and codeine, which can be fixed by selection in subsequent generations. Based on the present study, desirable accessions for specific alkaloids have been isolated and are used in different hybridization programmes aimed at the development of varieties rich in specific alkaloids for commercial cultivation in various specified locations of the states of India.

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