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Estimation of the extent of genetic divergence and clustering pattern among germplasm of Sarpgandha (*Rauvolfia serpentina*)

Rashmi Bakhla and Jai Kumar

Abstract
Keeping in view the importance of divergence study, a systematic research trial was undertaken to quantify extent of divergence of different Sarpgandha germplasm through its qualitative and quantitative traits at AICRP (M&APs) farm, BAU, Ranchi. Among the sources of genetic diversity in Sarpgandha germplasm, maximum percentage contribution was shown by seed yield/plant (51.33%) followed by inflorescence length (19.33%) and number of flower/inflorescence (11.67%). Seven clusters were formed, out of which cluster I consisted of 9 germplasm, followed by cluster II (8 germplasm) and cluster III (4 germplasm). Maximum inter cluster divergence was found between cluster III and cluster VI (13.59) followed by cluster II and cluster III (12.46) and cluster III and VII (10.44). So parents may be selected for hybridization program from these clusters i.e. cluster (III & VI), (II & III) and (III & VII).

Keywords: Sarpgandha, *Rauvolfia serpentina*, genetic divergence, germplasm, cluster

Introduction
Determining the amount, cause and nature of divergence present in the crops of interest is the first step towards any improvement work. The last few decades have witnessed the emergence of wide spread concern to solve the major problem of the erosion of genetic resources. The only realistic solution of the continuing loss of plant germplasm is the collection and systematic preservation of germplasm in gene resources centers with a wide representation of genetic resources of the species as practicable [19]. An essential prerequisite for a species to survive against environmental pressures is the availability of a pool of genetic diversity and in the absence of that extinction would appear inevitable [3]. Determining how much genetic diversity exists in a species and explaining this diversity in terms of its origin, organization and maintenance are thus of fundamental significance in the application of genetic principles to conservation.

In Sarpgandha, low genetic diversity is reported by various authors, which further inhibits the chances of improvement activities thus it is necessary to collect diverse germplasm of it from various eco-geographic regions, evaluate it, and select the parents for out crossing to improve the chances of selecting better segregants for various characters. Therefore, success in crop improvement programmes of Sarpgandha can be achieved through utilization of the broad genetic base of cultivated, as well as wild relatives and the diverse genetic materials may meet the ever-increasing demands of plant improvement. The assessment of genetic diversity using quantitative and qualitative traits in Sarpgandha is particularly important in many contexts because it is a self to often cross pollinated crop. In spite of the immense therapeutic value of Sarpgandha, lack of genetic information has lead to sequential problem for the selection of

divergent genotypes for crossing, as well as effective conservation and management. Thus it is necessary to collect diverse germplasm of it from various eco-geographic regions, evaluate it, and select the parents for out crossing to improve the chances of selecting better sergeant's for various characters.

Rauvolfia serpentina (L.) Benth. Ex Kurz. commonly known as Sarpgandha (Indian snakeroot), is critically endangered medicinal plant species. It is an important medicinal plant found in Indian subcontinent and south East Asian countries. It belongs to the family apocynaceae and its habit is evergreen, perennial, glabrous and erect under shrub, 60-90 cm in height and grows generally in the region with annual rainfall of 200-250 cm and up to an altitude of 1300-1400 m. The root system consists of a prominent, tuberous, soft tap-root, reaching a length of 30-50 cm in a 2 year old plant. Its diameter at the thickest portion varies from 1.2 to 2.5 cm. The root-bark, which constitutes 40-60% of the whole root, is rich in alkaloids. Breeding behavior is self to often cross-pollinated. The fresh roots emit a characteristic acrid aroma and are very bitter in taste. Sarpgandha is low-diverse, endangered and red-listed plant species because of over exploitation. Ruthless collection developed stress to its plant stand in natural habitat. Govt. of India prohibited its collection from wild. Keeping in view the importance of divergence study in Sarpgandha, a systematic research trial was undertaken to quantify extent of divergence of different Sarpgandha germplasm through its qualitative and quantitative traits at AICRP (M&APs) farm, Birsa Agricultural University, Ranchi with the objective was to estimate the extent of genetic divergence and clustering pattern among selected germplasm of Sarpgandha

Material and Methods

The experimental materials comprised of twenty five genotypes of Sarpgandha. Care was taken to maintain a geographical distance of about 8-10 km between each collection site in order to reduce genetic similarity in accessions and to minimize sampling error while interpreting the data. Germplasm collection of Sarpgandha was organized as an assemblage of seeds/cuttings collected through explorations and established in nursery at AICRP Research Farm of Birsa Agricultural University, Ranchi for transplantation purpose and for evaluation & characterization studies. While collecting plants from their natural habitats stratified random sampling method [9] was followed. Collected seed samples were germinated, raised in polytubes and maintained under identical growing conditions in experimental area and used for analyzing qualitative and quantitative variations. Forty five days old seedlings were transplanted in field at a spacing of 50 cm × 50 cm. Normal cultural practices such as weeding, hoeing and irrigation were given in the field. Experiment was laid out in Randomized Block Design with 25 treatments replicated thrice by following the procedure outlined by [12]. Number of seedlings per treatment was kept as 25.

Genetic diversity estimation of *Rauvolfia serpentina* germplasm: Genetic diversity in twenty five germplasm of Sarpgandha was estimated by multivariate analysis of genetic divergence of Mahalonobis "D^2" statistics [7]. Treating D^2 as the square of generalized distance, all germplasm were grouped into a number of clusters, according to the methods described by Tocher [15]. The replicated data of all the 25 germplasm were subjected to genetic divergence analysis. Variances and covariances for all the characters were calculated and a dispersion table was prepared. Wilk's criteria were used to test the significance differences in mean values of all the characters. Using the common error dispersion matrix, the D^2 value was calculated for each pair of germplasm among all possible combination. Average intra and inter cluster distances were calculated for all the clusters. For calculating the inter-cluster distance, two clusters were taken at a time and total distance between the populations falling in these two clusters was calculated.

Data recorded for genetic divergence studies were% contribution of factors towards genetic divergence, average intra & inter cluster D^2 value and mean performance of clusters. Statistical analysis of the data was carried out and the relative contribution of different twenty characters to the total D^2 between each pair of germplasm was calculated based on the magnitude of the D^2 value due to each character. Following parameters of genetic diversity analysis of Sarpgandha was done. a. Sources and contribution of different traits towards genetic divergence of Sarpgandha germplasm, b. Clustering pattern of Sarpgandha germplasm based on quantitative traits, c. Average intra and inter cluster distance and d. Mean performance of clusters for quantitative traits

Results and Discussion

Genetic variability in Sarpgandha germplasm: Contribution of growth, yield and reproductive parameters towards genetic divergence in Sarpgandha germplasm is presented in Table 1.

Sources and contribution of different characters towards genetic divergence in Sarpgandha germplasm: Parameters such as seed yield/plant (51.33%), followed by inflorescence length (19.33%) and number of flowers/inflorescence (11.67%) contributed maximum towards genetic diversity in Sarpgandha germplasm. Minimum contribution was shown by stem diameter & number of leaves/plant (1.33%) followed by plant height (1.67%), root length (2.00%) and number of inflorescence/plant & number of fruits/inflorescence both (3.00%). Number of primary branches/plant and root diameters had not any contribution (0.00%) towards genetic diversity of Sarpgandha.

Table 1: Sources of genetic diversity in Sarpgandha germplasm with their percentage contribution

Sl. No.	Source (Growth parameters)	Percentage contribution
1.	Plant height	1.67
2.	Stem diameter	1.33
3.	Number of primary branches/plant	0.00
4.	No. of leaves/plant	1.33
5.	Inflorescence length	19.33
6.	No. of inflorescences/plant	3.00
7.	No. of flowers/inflorescences	11.67
8.	No. of fruits/inflorescences	3.00
9.	Seed yield/plant	51.33
10.	Root length	2.00
11.	Root diameter	0.00
12.	Dry root yield/plant	5.33

Dry root yield per plant, root length and plant height were very potent in contributing towards divergence in Ashwagandha [4]. Characters like number of fruits per plant, average fruit weight, plant height and fruit yield contributed maximum towards genetic divergence in Tomato [17]. Leaf number contributed most toward divergence (15.8%),

followed by finger number, length and width (each with at least 13% contribution) and leaf length (12.1%) in Kalmegh [5]. Number of pods per plant who contributed maximum genetic divergence in Kalmegh [10].

Average intra and inter-cluster D^2 values among Sarpgandha germplasm: Table 2 represents average intra and inter-cluster D^2 values among twenty five genotypes of Sarpgandha for growth, reproductive and yield. Intra-cluster average D^2 values ranged from 0.00 to 5.32 for selected characters. In growth, yield and reproductive characters, cluster III showed maximum intra D^2 values (5.32) with four germplasm. Clusters IV, V, VI and VII showed minimum intra D^2 value (0.00) each with one genotype. In general, mean inter cluster distance was followed the trend in decreasing order as cluster III (5.32) > cluster II (5.25) > cluster I (4.11) > cluster IV = cluster V = cluster VI = cluster VII (0.00).

In this investigation, inter-cluster average D^2 values for growth, yield and reproductive characters was maximum (13.59) between cluster III and VI followed by 12.46 between clusters II and III and 10.44 between clusters III and VII indicating wide divergence among the clusters. Minimum inter cluster values for growth, yield and reproductive characters was recorded between clusters VI and VIII (5.29) followed by between clusters I and V (5.79) and clusters I and IV (5.87) indicating the close relationship among the genotypes included in these two clusters. Average intra and inter cluster D^2 values among twenty five genotypes revealed that cluster IV, V, VI and VII showed no intra cluster D^2 value as it had only one genotype each. The genotypes included in the diverse clusters namely IV, V, VI and VII hold good promise as parents for obtaining potential hybrids and thereby creating greater variability of these characters to improve the yield.

Range of genetic diversity of Sarpgandha in terms of genetic distance from 0.596 to 0.928 with mean value of 0.820 [11]. Significant differences in all the metric traits and sufficient inter-cluster distances in Kalmegh [6]. D^2 values for all the pairs of accessions ranged from 0.01 to 76.98 thereby indicating considerable diversity in the Kalmegh material. High inter-cluster D^2 values recorded high between cluster II and III and cluster III and VI in Safed musli indicated the possibility of raising transgressed hybrids from cross hybridization programs using divergent parents of these four clusters [5]. Through D^2 analysis, there was considerable divergence among the genotypes of Ashwagandha [13]. Maximum intra cluster distance was observed in cluster-IV (10.61) and minimum in cluster-I (7.91), while inter cluster distance was found to be maximum between cluster VI and cluster V (24.34) and minimum between cluster II and cluster III (9.16).

Table 2: Average intra and inter-cluster divergence among Sarpgandha germplasm

Clusters	I	II	III	IV	V	VI	VII
I	4.11						
II	6.74	5.25					
III	8.68	12.46	5.32				
IV	5.87	8.37	7.37	0.00			
V	5.79	7.11	10.15	7.82	0.00		
VI	8.32	6.50	13.59	8.54	7.88	0.00	
VII	7.00	6.97	10.44	6.08	7.25	5.29	0.00

The D^2 statistics showed that there was adequate diversity among the genotypes with D^2 values ranging from 8.47 to 99.38. On the basis of D^2 values, 20 genotypes were grouped into 6 different clusters.

Clustering pattern of twenty five germplasm of Sarpgandha: Details of clustering pattern of twenty five germplasm of Sarpgandha are presented in Table 3. 25 germplasm of Sarpgandha were grouped into seven clusters. Cluster I consisted of maximum nine germplasm, followed by cluster II (eight germplasm), cluster III (4 germplasm). Cluster IV, V, VI and VII consisted of only one germplasm of Sarpgandha. Overall genetic variation in a species/population is primarily due to the dynamic balance between genetic drift and gene flow [2]. Genetic diversity is primarily due to the dynamic balance between genetic drift and gene flow [1].

Table 3: Clustering pattern of twenty five germplasm types of Sarpgandha based on genetic divergence among growth and yield parameters

Sl. No.	Cluster	No. of Germplasm	Germplasm
1	I	9	BRS_6, BRS_7, BRS_8, BRS_9, BRS_{10}, BRS_{11}, BRS_{13}, BRS_{19}, BRS_{22}
2	II	8	BRS_1, BRS_4, BRS_5, BRS_{15}, BRS_{16}, BRS_{17}, BRS_{18}, BRS_{24}
3	III	4	BRS_{12}, BRS_{14}, BRS_{21}, BRS_{23}
4	IV	1	BRS_{25}
5	V	1	BRS_{20}
6	VI	1	BRS_2
7	VII	1	BRS_3

Graph 1 represents cluster diagram of 25 germplasm of Sarpgandha, cluster I contains maximum number of germplasm (nine), followed by cluster II (eight), cluster III (four), and cluster IV, V, VI & VII were monogenotypic (one each). Study of clustering pattern indicated that genotypes BRS_2, BRS_3, BRS_{20} and BRS_{25} were quite different to other varieties, distributed in divergent clusters. Highest genetic distance for growth, yield and reproductive characters were observed between cluster III and VI (13.59). Clustering pattern of the genotype showed that the genetic relationship and diversity of Sarpgandha germplasm are not closely associated with their growth habit, geographical origin and ecological distribution. Genotypes present in the clusters namely IV, V, VI and VII hold good promise as parents for obtaining potential hybrids and create greater variability to improve the yield. High inter cluster distances were main cause of heterogeneity in composition of clusters. The genetic distinctness of gene pools from different regions species may be associated with reproductive isolation, due to wide geographic separation, as well as to non-overlapping ecological adaptation [20, 21, 14].

Genetic divergence among 37 accessions of Ashwagandha was quantified for six metric traits and classified in eight clusters showing substantial divergence [8]. Cluster I was largest comprising 14 genotypes followed by cluster II, III & IV having 13, 4 and 2 genotypes respectively, and rest having single genotype. Nature and magnitude of genetic divergence in thirty five chilli genotypes of different geographical origin using Mahalanobis D^2 statistics and grouped them into six clusters [18]. The cluster-II was the largest with 16 genotypes followed by cluster – III with six and cluster-V with five genotypes.

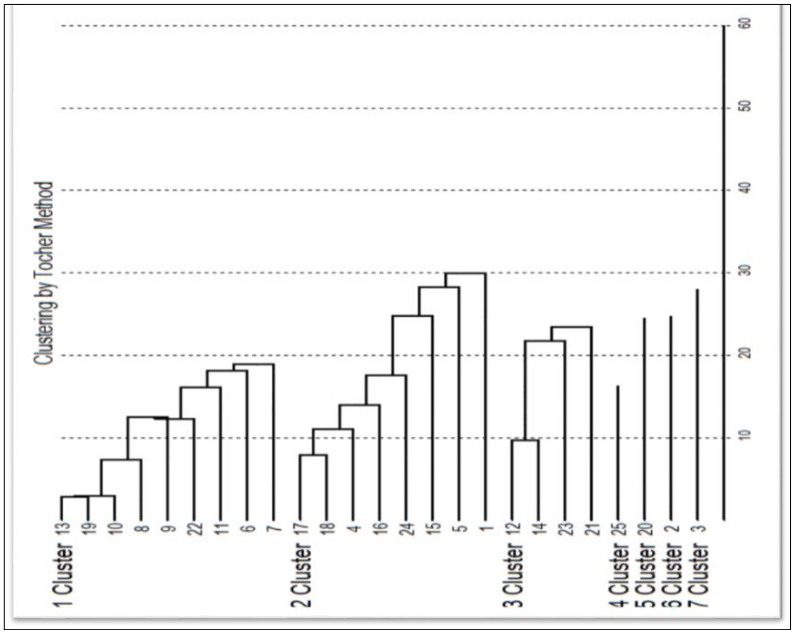

Fig 1: Clustering pattern of twenty five genotypes of Sarpgandha based on genetic divergence among growth and yield parameters

Overall it can be concluded that the germplasm included in present investigation were grouped in different clusters and did not follow the geographic distribution. Study of clustering pattern indicated that germplasm related by pedigree fell in either same cluster or in cluster with high inters cluster distances. BRS_2, BRS_3, BRS_{20} and BRS_{25} were quite different to other varieties by pedigree. These four germplasm were distributed in divergent clusters probably due to differences in their pedigree. Above findings indicated that use of parents selected from the same cross or from a cross involving a common parent should be avoided in hybridization. Choice of parents for hybridization should be based on genetic diversity rather than geographical diversity [16].

Mean performance of clusters for growth and yield characters in Sarpgandha: Table 4 represents mean performance of clusters for growth and yield characters in Sarpgandha. For plant height, cluster VI had the highest mean values (67.67 cm) followed by cluster IV (65.67 cm), while its minimum value was recorded for cluster V (33.33 cm) followed by cluster I (51.20 cm). For stem diameter, cluster IV had the highest mean values (9.09 mm) followed by cluster VI (8.97 mm), while its minimum value was recorded for cluster VII (7.71 mm) followed by cluster I (8.50 mm). For number of primary branches/plant, cluster VII had the highest mean values (6.67) followed by cluster IV (5.67), while its minimum value was recorded for cluster V (3.67) followed by cluster II (4.54). For number of leaves/plant, cluster VII had the highest mean values (65.00) followed by cluster III (52.08), while its minimum value was recorded for cluster V (24.33) followed by cluster II (37.14). For inflorescence length, cluster II had the highest mean values (88.09 mm) followed by cluster VII (87.15 mm), while its minimum value was recorded for cluster I (72.20 mm) followed by cluster VI (76.16 mm).

For number of inflorescence/plant, cluster VI had the highest mean values (13.67) followed by cluster VII (13.00), while its minimum value was recorded for cluster IV (4.00) followed by cluster I (4.96). For number of flowers/inflorescence, cluster III had the highest mean values (62.08) followed by cluster II (58.75), while its minimum value was recorded for cluster IV (16.33) followed by cluster VI (21.00). For number of fruits/inflorescence, cluster III had the highest mean values (54.67) followed by cluster II (53.00), while its minimum value was recorded for cluster IV (8.67) followed by cluster VI (15.33). For seed yield/plant, cluster III had the highest mean values (20.33 g) followed by cluster IV (13.31 g), while its minimum value was recorded for cluster VI (2.09 g) followed by cluster II (4.34 g). For root length, cluster VI had the highest mean values (88.17 cm) followed by cluster IV (86.63 cm), while its minimum value was recorded for cluster V (68.30 cm) followed by cluster II (76.96 cm). For root diameter, cluster VII had the highest mean values (17.07 mm) followed by cluster IV (14.94 mm), while its minimum value was recorded for cluster V (13.68 mm) followed by cluster VI (13.93 mm). For dry root yield/plant, cluster III had the highest mean values (75.29 g) followed by cluster IV (75.16 g), while its minimum value was recorded for cluster V (42.85 g) followed by cluster VII (59.82 g).

Among genetic diversity of Kalmegh from 53 accessions belonging to 5 ecogeographic regions of India based on morphochemical markers, maximum mean dry herbage yield/plant was shown by cluster V (50.59g) and minimum by cluster III (33.36g) [6]. Maximum mean leaf/stem ratio was shown by cluster IV (0.48) and minimum by cluster II and V (0.35). Maximum mean plant height was shown by cluster III (73.18 cm) and minimum by cluster I (57.99 cm). Maximum mean leaf length was shown by cluster V (5.09 cm) and minimum by cluster I (4.79 cm). Maximum mean leaf width was shown by cluster III, IV and V (1.64 cm) and minimum by cluster I (1.52 cm). Maximum mean plant spread was shown by cluster V (54.90 cm) and minimum by cluster IV (41.33 cm). Maximum mean number of leaves/plant was shown by cluster IV (419) and minimum by cluster II (283).

Table 4: Mean performance of clusters for growth and yield characters in Sarpgandha

Clusters	Cluster Means											
	Plant height (cm)	Stem diameter (mm)	Number of primary branches/plant	No. of leaves/plant	Inflorescence length	No. of inflorescences/plant	No. of flowers/inflorescences	No. of fruits/inflorescences	Seed yield/ plant	Root length	Root diameter	Dry root yield/ plant
I	51.20	8.50	4.56	44.41	72.20	4.96	56.93	51.00	8.77	77.77	14.65	69.48
II	54.80	8.78	4.54	37.14	88.09	8.04	58.75	53.00	4.34	76.96	14.72	70.82
III	53.17	8.90	5.08	52.08	76.73	7.25	62.08	54.67	20.33	77.03	14.43	75.29
IV	65.67	9.09	5.67	50.00	82.14	4.00	16.33	8.67	13.31	86.63	14.94	75.16
V	33.33	8.72	3.67	24.33	76.89	7.33	49.00	40.00	8.84	68.30	13.68	42.85
VI	67.67	8.97	5.33	49.33	76.16	13.67	21.00	15.33	2.09	88.17	13.93	63.62
VII	53.13	7.71	6.67	65.00	87.15	13.00	21.67	15.67	8.10	78.27	17.03	59.82

Conclusion

So to conclude regarding estimation of extent of genetic divergence and clustering pattern among selected germplasm of Sarpgandha, following inferences can be drawn. Clustering pattern indicated 7 clusters formed out of which cluster I consisted of 9 germplasm, followed by cluster II (8 germplasm) and cluster III (4 germplasm). Cluster IV, V, VI and VII consisted of only one germplasm each. Maximum inter cluster divergence was found between cluster III and cluster VI (13.59) followed by cluster II and cluster III (12.46) and cluster III and VII (10.44). So parents may be selected for hybridization program from these clusters i.e. cluster (III & VI), (II & III) and (III & VII). Minimum inter cluster divergence was noticed between cluster VI and cluster VII (5.29). Mean intra cluster divergence was found maximum in cluster III (5.32) and minimum in cluster IV, V, VI and VII (0.00).

As regards to percentage contribution of quantitative traits towards its genetic divergence, following inferences can be drawn. Maximum genetic divergence was shown by seed yield/plant (51.33%), inflorescence length (19.33%) & number of flowers /inflorescences (11.67%). Number of primary branches /plant & root diameter shown 0% contribution towards genetics divergence of Sarpgandha while low percentage contribution towards genetics divergence was shown by plant height (1.67%), stem diameter (1.33%), number of leaves/plant (1.33%) and root length (2.00%).

Cluster means was recorded maximum for plant height (cluster VI, 67.67 cm), stem diameter (cluster IV, 9.09 mm), number of primary branches/plant (cluster VII, 6.67), number of leaf/plant (cluster VII, 65), inflorescence length (cluster II, 8.81cm), number of inflorescence/plant (cluster VI 13.67), number of flowers/inflorescence (cluster III, 62.08), number of fruits/inflorescence (cluster III, 54.67), seed yield/plant (cluster III, 20.33 g), root length (cluster VI, 88.17 cm), root diameter (cluster VII, 17.03 mm) and dry root yield/plant (cluster III, 75.39 g).

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