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Studies on genetic diversity in Indian mustard (*Brassica Juncea* Czern & Coss) for morphological characters under changed climate in the mid-hills of Himalayas

Arpna Kumari and Vedna Kumari

Abstract

In the present study, we investigated the genetic diversity pattern in thirty one *Brassica juncea* genotypes, using morphological traits. The genotypes were analysed for fifteen morphological traits in randomized complete block design replicated three times in two consecutive years [*rabi* 2008-09 (Env.I) and *rabi* 2009-10 (Env.II)]. Field data of two consecutive years were initially subjected to analysis of variance. Highly significant genotypic differences were found in the combined analysis of variance for days to flower initiation, days to 50 per cent flowering, days to 75 per cent maturity, plant height, siliqua length, 1000- seed weight and harvest index, confirming the possibility of improving these traits through selection. Moreover, genotypes responded differently to changes in the environmental conditions at the two locations as $G \times E$ interaction mean squares were highly significant ($P \leq 0.01$) for all the traits except for days to 50 per cent flowering, days to 75 per cent maturity, plant height, number of primary branches per plant, siliqua length and 1000- seed weight. The phenotypic divergence and relative importance were estimated by multivariate analysis. The cluster analysis based on Tocher's method classified the genotypes into four major groups of different sizes. Cluster I was the largest with 28 genotypes or constituting closer to 90 % of the total population, while cluster II, III and IV had one genotype each. The intra- cluster distance was comparable for cluster I ($D^2 = 1.14$), while for clusters II, III and IV, intra-cluster distance was zero as the clusters were constituted by single genotype each. The maximum inter-cluster distance was found between clusters II and III ($D^2 = 1.81$). The second most divergent clusters were III and IV ($D^2 = 1.78$). Among the traits, 1000- seed weight contributed maximum (17.63 %) to genetic divergence followed by days to flower initiation (16.13 %) and siliqua length (12.26 %). Principal component analyses based on phenotypic data identify six most informative principal components explaining at least 80.92 % of the total variability, in particular PC1 contributing with 28.02 %, PC2 with 17.85 %, PC3 with 12.89 %, PC4 with 8.82 %, PC5 with 7.86 % and PC6 with 5.48 %. The principal component analysis largely confirmed the grouping of the genotypes obtained through cluster analysis.

Keywords: *Brassica juncea*, Indian mustard, Genetic diversity, Mahalanobis D^2 -statistics, Principal Component analysis

1. Introduction

Brassica species, commonly called as rapeseed-mustard, are the third most important oilseed crops of the world after soybean and palm. China, India, Canada, Japan and Germany are the major rapeseed-mustard growing countries. These are the second most important oilseed crops of India, next to soybean. India is one of the largest rapeseed-mustard growing country occupying first position with 20.23% area and second position with 11.7% share to the global production (USDA 2017) [16]. Four oleiferous *Brassica* species viz. *Brassica juncea*, *B. napus*, *B. rapa* and *B. carinata* are cultivated in about 6.39 million hectares area and produce 7.41 million tons in India (Kumar *et al.* 2012) [7]. *B. juncea* ($2n=36$, AABB genome), an allopolyploid commonly called as Indian mustard, contributes more than 80% to the total rapeseed-mustard production in the country and is an important component in the oilseed sector. It is known to be more drought tolerant and shattering resistant than *B. napus* and *B. rapa*, therefore, has an enormous cultivation potential in semi-arid areas.

With the increasing population and improving life standards, *per capita* oil consumption has increased tremendously. To meet out the present oil requirements, there is an urgent need to increase the yield potential of *B. juncea* through genetic interventions. The maximum utilization of any species for breeding and its adaptation to different environments depend on the level of genetic diversity it holds. Genetic distance among parents may be attributed to their differences for number of genes and their functional relations in a given environment.

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Evaluation of genetic divergence and relatedness among breeding materials has significant implications for the improvement of crop plants. Knowledge on genetic diversity in *B. juncea* could help breeders and geneticists to understand the structure of germplasm, predict which combinations would produce the best offsprings (Hu *et al.* 2007) [6], and facilitate to widen the genetic basis of breeding material for selection (Qi *et al.* 2008) [12].

Genetic diversity among individuals or populations can be determined using morphological, biochemical and molecular approaches (Mohammadi and Prasanna 2003) [9]. Assessment of genetic diversity in *B. juncea* using phenotypic characters has previously been done by many researchers (Vaishnav *et al.* 2006, Alie *et al.* 2009, Singh *et al.* 2010) [17, 1, 14].

Therefore, the present study was undertaken to estimate the genetic diversity of 31 *B. juncea* genotypes of diverse geographic origin and explore potential to evaluate the relationship of these genotypes based on quantitative trait data. Genetic diversity will further help in identifying genetically potential genotypes, which then can be utilized in creating valuable selectable variation.

2. Materials and Methods

2.1 Plant material and experimental site

A total of 31 genotypes of *Brassica juncea* obtained from local, indigenous and exotic sources were used in the present study (Table 1). All the genotypes, including three checks, *viz.*, RCC-4, RL-1359 and Varuna were raised at the experimental farm of the Department of Crop Improvement, Chaudhary Sarwan Kumar Himachal Pradesh Krishi Vishvavidyalaya, Palampur (H.P.), India, during two consecutive years of *rabi* season, 2008-09 (Env.I) and 2009-10 (Env.II), for recording the morphological data. The experimental site is located at 1290.8 m amsl and at 32°8' N latitude and 76°3' E longitude. Agro-climatically, the location represents the mid-hill zone of Himachal Pradesh (Zone-II) and is characterized by humid sub-temperate climate with high rainfall (2,693 mm). The soils are clay loam to silty clay loam in texture. The reaction of soil is acidic with pH ranging from 5.0 to 5.6. The more information on locations and climatic conditions are given in Table 2 and Figures (1& 2).

2.2 Experimental design and layout

At two environments, experimental layout was a randomized complete block design with three replications. Plot sizes were 2.7 m² (3 rows, 3 m long and 30 cm row spacing) and 2.25 m² (3 rows, 2.5 m long and 30 cm row spacing). A pre-sowing irrigation was given to ensure proper germination. Irrigation was given whenever required and regular weeding was done to keep the trial free from weeds.

2.3 Recording of observations

Data were recorded on fifteen different morphological traits namely days to flower initiation, days to 50 per cent flowering and days to 75 per cent maturity, plant height, number of primary branches per plant, number of secondary branches per plant, siliquae per plant, length of main shoot, siliquae on main shoot, siliqua length, seeds per siliqua, 1000-seed weight, seed yield per plant, biological yield per plant and harvest index. Five competitive plants were tagged randomly from each genotype in each replication for recording field observations for all the traits except for days to flower initiation, days to 50 per cent flowering and days to 75 per cent maturity, which were observed on plot basis during both

the years. Thousand seed weight was measured from a random sample of 1000 seeds from the total amount of seed obtained from each of the five selected plants per plant. Harvest index was calculated by dividing seed yield per plant by biological yield per plant.

2.4 Statistical analysis

The data recorded for each genotype at each environment were subjected to statistical analysis. An analysis of variance (ANOVA) was conducted in each environment to test significant differences among genotypes (Panse and Sukhatme 1985) [11]. Homogeneity of error variance tests were conducted to determine if data from individual environments (E) could be pooled to evaluate G × E interaction using a combined ANOVA as per (Verma *et al.* 1987) [18]. The Homogeneity of error variances were tested with F-test or the 'variance ratio' test as described by (Gomez and Gomez 1984 [5]). For the combined analysis, variation was partitioned into relevant sources of variation to test for differences among genotypes and for the presence of G × E interaction. The phenotypic divergence among the accessions was estimated by the multivariate techniques, as follow: Tocher's cluster analysis as described by (Rao 1952) [13], using Mahalanobis D²-statistics Mahalanobis (1936) [8] to measure the genetic distance. To better understand the correlation between all characters studied with seed yield, principal component analysis (PCA) was performed using a matrix generated from the mean morphological data, followed by cluster analysis by K-means method and the Euclidean distance.

3. Result and Discussion

3.1 Univariate analysis of variance (ANOVA)

Homogeneity of variance tests indicated homogeneous error variance for each trait in each of the two environments and allowed for a combined, across environment analysis. There was a significant effect of environment for all the traits (ANOVA not shown). Highly significant genotypic differences were found in the combined analysis of variance for days to flower initiation, days to 50 per cent flowering, days to 75 per cent maturity, plant height, siliqua length, 1000- seed weight and harvest index, confirming the possibility of improving these traits through selection. Significant genetic variation has also been reported by (Vaishnav *et al.* 2006, Alie *et al.* 2009, Yadava *et al.* 2009, Singh *et al.* 2010, Vinu *et al.* 2013, Chandra *et al.* 2018) [17, 1, 23, 14, 20, 3] on metric traits in *B. juncea*. Moreover, genotypes responded differently to changes in the environmental conditions at the two locations as G × E interaction mean squares were highly significant ($P \leq 0.01$) for all the traits except for days to 50 per cent flowering, days to 75 per cent maturity, plant height, number of primary branches per plant, siliqua length and 1000- seed weight. Similar observations were reported earlier in Indian mustard (Verma *et al.* 2008, Singh *et al.* 2010, Yadava *et al.* 2011) [19, 14, 22].

3.2 Multivariate analysis

The genotypes were grouped into four diversity classes (Table 3 & Figure 3), different members within a cluster being assumed to be more closely related in terms of the traits under consideration with each other than those members in different clusters. Cluster I was the largest with 28 genotypes or constituting closer to 90 % of the total population, while cluster II, III and IV had one genotype each. The cluster I constituted intermediate genotypes for yielding potential and

seed size (Table 4). Cluster II constituted the best yielding genotypes with large seed size, siliquae on main shoot and length of main shoot, and least in number of days to 50 per cent flowering and siliquae per plant. Cluster III and cluster IV constituted superior genotypes for most of the traits including number of primary branches per plant, secondary branches per plant, siliquae per plant, seeds per siliqua, biological yield per plant and harvest index, the shortest in plant height, and least in days to flower initiation and days to 75 per cent maturity. Similar findings were reported by earlier workers (Tahira *et al.* 2013, Gohel and Mehta 2014, Anushree and Anil Pandey 2017, Chandra *et al.* 2018) ^[15, 4, 2, 3].

The pairwise generalized squared distances (D^2) among the four clusters presented in Table 3 and Figure 5. The intra-cluster distance was comparable for cluster I ($D^2 = 1.14$), while for clusters II, III and IV, intra-cluster distance was zero as the clusters were constituted by single genotype each. Since, the intra-cluster distance was low, therefore, it is logical to attempt crosses between the genotypes falling in different clusters based on inter-cluster distances. The maximum inter-cluster distance was found between clusters II and III ($D^2 = 1.81$). The second most divergent clusters were III and IV ($D^2 = 1.78$). The genetic distance between clusters II and IV ($D^2 = 1.66$), clusters I and III ($D^2 = 1.53$) and clusters I and IV ($D^2 = 1.45$). The inter-cluster distance was minimum between clusters I and II ($D^2 = 1.41$), suggesting close relationship among the genotypes. Among the traits, 1000-seed weight contributed maximum (17.63 %) to genetic divergence followed by days to flower initiation (16.13 %) and siliqua length (12.26 %) (Table 5).

3.3 Principal component analysis (PCA)

Principal component analysis is a multivariate technique was used in several studies to access the interrelationship between various traits analysed with interesting traits and to clustering the genotypes. Hence, in the present study PCA was used to illustrate the correlation between the morphological traits. So, a matrix of mean values for two years was used for analysis. Correlation between the characters studied and first six principal components is shown in Table 6 and Figure 4. In principal component analysis to estimate the relative contribution of traits towards the variation in the 31 genotypes, the first six principals components accounted for 80.92 % of the entire diversity among the genotypes for all the traits investigated. PC1, PC2, PC3, PC4, PC5 and PC6, respectively, explained 28.02, 17.85, 12.89, 8.82, 7.86 and 5.48 % of the total variability (Table 6 & Figure 4). Similar findings reported by (Saleem *et al.* 2017) ^[10], Wang *et al.* (2009) ^[21] also used PCoA to delineate and visualise 405

individuals and 48 varieties of *B. napus* into four cluster. The first principal component accounted for 28.02 % of total variation, indicating that this axe represent the majority of the variation for the character studied. It was mainly determined by the days to flower initiation and biological yield per plant. Hence it is correlated negatively to the siliqua length. PC2 accounted for 17.85% of the variance existing in the genotypes. This variation among the genotypes contributed by days to flower initiation and plant height. The third PC was associated with siliqua length, length of main shoot and seeds per siliqua. The fourth PC was positively associated with seed yield per plant and negatively associated with number of primary branches per plant and secondary branches per plant. The principal component analysis largely confirmed the grouping of the genotypes obtained through cluster analysis.

Table 1: List of *Brassica* genotypes and their source used in the study

Genotype	Source
Vardan	Kanpur
03-218	H.P.
HPMM-03-108	H.P.
03-143	H.P.
RCC-4	H.P.
OMK-2	H.P.
NRC-1	Rajasthan
NRC-2	Rajasthan
NRC-17	Rajasthan
PusaJaikisan	New Delhi
03-456	H.P.
Heera	Exotic
RL-1359	Ludhiana
OMK-5-1	H.P.
OMK-1	H.P.
OMK-2-21	H.P.
OMK-3	H.P.
OMK-3-29	H.P.
IC-355309	NBPGR, New Delhi
IC-355331	NBPGR, New Delhi
IC-355337	NBPGR, New Delhi
Geeta	Haryana
IC-355421	NBPGR, New Delhi
Bawal-151	Haryana
Varuna	Kanpur
OMK-5-2	H.P.
RH-8544	Hisar
Nav Gold	Rajasthan
OMK-5-3	H.P.
OMK-5-4	H.P.
Zem-1	Exotic

Table 2: Descriptions of environments where trials were conducted during 2008–10

Location	Cropping season	Month	Temperature (°C)		Rainfall (mm)	Relative Humidity (%)	Rainy Days (No.)	Solar radiation (MJ m ⁻² day ⁻¹)
			Max	Min				
Palampur (E-I)	rabi (2008-09)	Oct.	25.2	13.1	65.4	73	5	8.0
		Nov.	22.2	8.6	0.0	60	0	9.0
		Dec.	20.5	7.6	9.2	58	2	7.5
		Jan.	17.5	6.5	56.4	72	9	5.3
		Feb.	19.0	7.5	32.0	66	5	7.0
		March	22.7	10.3	89.2	58	5	6.2
		April	26.4	13.8	65.0	55	6	8.1
Palampur (E-II)	rabi (2009-10)	Oct.	25.6	11.6	33.9	80.48	4	9.3
		Nov.	20.8	7.6	69.4	81.54	5	7.1
		Dec.	18.0	5.2	0.0	75.54	0	5.8

	Jan.	18.3	4.9	25.2	76.49	2	7.1
	Feb.	18.3	6.2	120.6	82.66	6	6.2
	March	25.6	12.4	26.0	61.40	3	8.1
	April	30.3	15.7	27.9	48.30	5	8.1

Table 3: Intra- (bold) and inter-cluster divergence (D^2 values) among four clusters of linseed

Clusters	I	II	III	IV	Genotypes included in clusters
I (28)	1.31 (1.14)	1.98 (1.41)	2.35 (1.53)	2.09 (1.45)	RL-1359, OMK-3, NRC-1, OMK-3-29, 03-143, Varuna, Bawal-151, RH-8544, OMK-2-21, OMK-2, RCC-4, OMK-5-2, Zem-1, NRC-2, IC-355337, Nav Gold, NRC-17, IC-355331, IC-355421, 03-218, OMK-5-1, IC-355309, PusaJaikisan, Vardan, OMK-5-3, OMK-5-4, 03-456, OMK-1
II (1)		0.00 (0.00)	3.26 (1.81)	2.77 (1.66)	Geeta
III (1)			0.00 (0.00)	3.18 (1.78)	Heera
IV (1)				0.00 (0.00)	HPMM-03-108

Table 4 Cluster means for 30 genotypes studied for fifteen quantitative traits in pooled over the environments

Characters	Clusters I	II	III	IV	Mean	Minimum	Maximum
Days to flower initiation	60.24	58.00	66.00	55.67	59.98	55.67	66.00
Days to 50 % flowering	70.30	70.00	78.33	73.50	73.03	70.00	78.33
Days to 75 % maturity	148.07	149.83	151.83	145.50	148.81	145.50	151.83
Plant height	148.51	148.17	169.20	117.57	145.86	117.57	169.20
No. of primary branches/ plant	6.07	6.23	6.17	6.60	6.27	6.07	6.60
No. of secondary branches/ plant	17.79	18.50	20.77	17.43	18.62	17.43	20.77
Siliqueae/ plant	229.56	220.97	264.47	241.80	239.20	220.97	264.47
Length of main shoot	53.56	60.20	54.73	52.60	55.27	52.60	60.20
Siliqueae on main shoot	38.44	40.77	38.27	39.57	39.26	38.27	40.77
Siliquea length	4.71	5.03	4.08	5.36	4.80	4.08	5.36
Seeds/ siliquea	12.06	12.00	10.03	16.27	12.59	10.03	16.27
1000- seed weight	3.40	4.83	2.94	2.69	3.47	2.69	4.83
Seed yield/ plant	9.19	13.93	8.24	7.78	9.79	7.78	13.93
Biological yield/ plant	59.77	79.08	87.17	42.82	67.21	42.82	87.17
Harvest index	15.79	16.91	9.39	18.67	15.19	9.39	18.67

Table 5: Percent contribution of different quantitative traits toward genetic diversity in pooled over the environments

Characters	Times ranked 1 st	Contribution (%)
Days to flower initiation	75	16.13
Days to 50 % flowering	6	1.29
Days to 75 % maturity	5	1.08
Plant height	22	4.73
Number of primary branches/ plant	4	0.86
Number of secondary branches/ plant	45	9.68
Siliqueae/ plant	21	4.52
Length of main shoot	16	3.44
Siliqueae on main shoot	1	0.22
Siliquea length	57	12.26
Seeds/ siliquea	20	4.30
1000-seed weight	82	17.63
Seed yield/ plant	48	10.32
Biological yield/ plant	37	7.96
Harvest index	26	5.59

Table 6: Eigenvectors and accumulated variation of the first six components (PC) from the morphological correlation matrix derived from 30 *brassica* genotypes in pooled over the environments

Parameter	PCA1	PCA2	PCA3	PCA4	PCA5	PCA6
Percentage variance (%)	28.02	17.85	12.89	8.82	7.86	5.48
Cumulative variance (%)	28.02	45.88	58.77	67.59	75.45	80.93
Traits	Latent vectors					
Days to flower initiation	0.45	0.30	0.26	0.23	0.25	0.03
Days to 50 % flowering	0.29	-0.07	0.27	-0.15	-0.33	0.35
Days to 75 % maturity	0.13	0.24	-0.21	0.07	-0.30	-0.19
Plant height	0.30	0.28	-0.09	0.31	0.00	0.05
Number of primary branches/plant	-0.01	-0.03	0.05	-0.34	-0.04	0.20
Number of secondary branches/plant	0.03	0.11	-0.29	-0.56	-0.31	-0.08

Siliqueae/plant	-0.14	-0.20	-0.25	0.23	0.30	0.23
Length of main shoot	-0.11	0.15	0.37	0.03	-0.04	-0.23
Siliqueae on main shoot	0.08	0.02	0.17	-0.15	0.15	0.21
Siliquea length	-0.36	-0.06	0.59	-0.03	-0.12	-0.37
Seeds /siliquea	0.01	-0.24	0.30	-0.10	-0.03	0.56
1000-seed weight	-0.34	0.62	-0.07	-0.16	-0.02	0.29
Seed yield/plant	-0.32	-0.01	-0.04	0.53	-0.60	0.27
Biological yield/plant	0.35	0.24	0.19	0.01	-0.26	-0.03
Harvest index	0.31	-0.44	-0.12	0.02	-0.29	-0.19

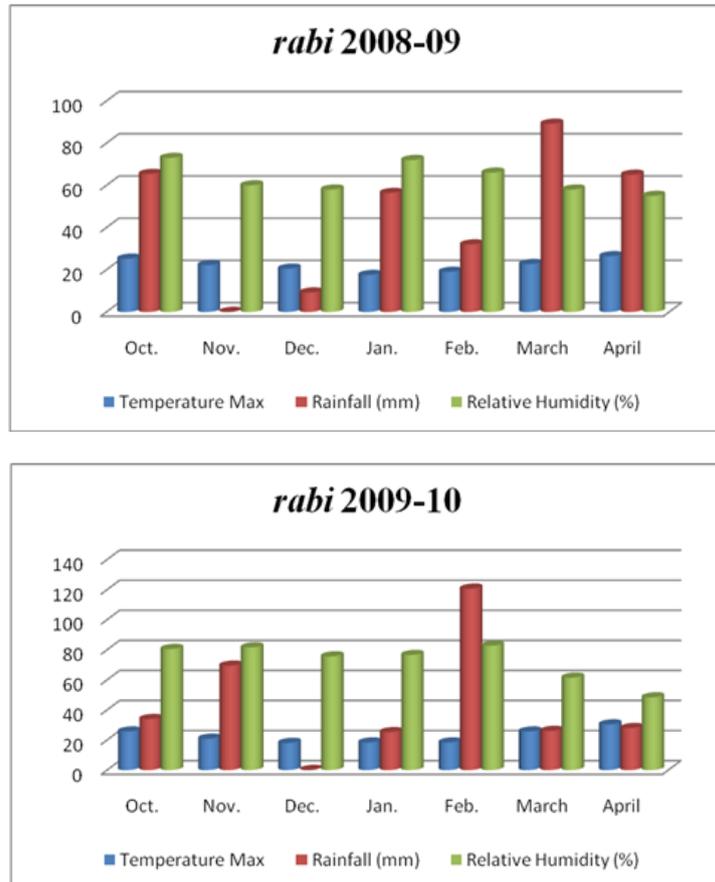


Fig 1& 2: Meteorological data for trial sites average maximum temperature, total rainfall and average relative humidity by month during the growing season 2008–2010

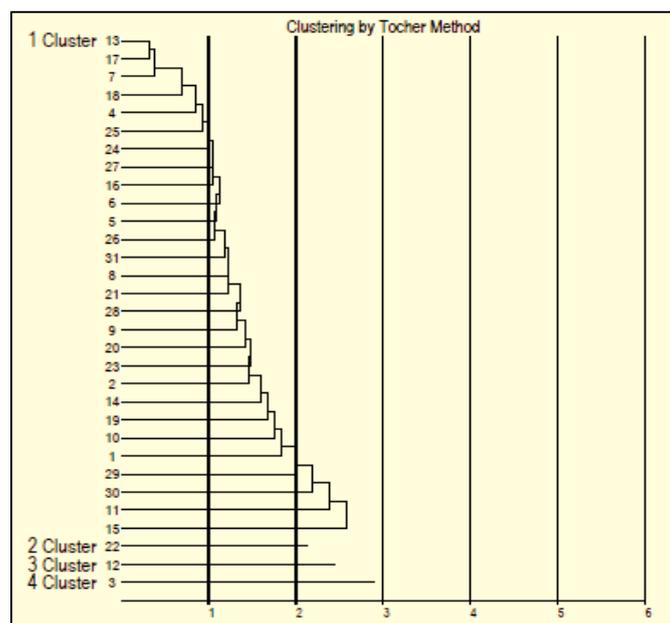


Fig 3: Dendrogram showing grouping of 31 *Brassica* genotypes generated using D² cluster analysis (Tocher's method)

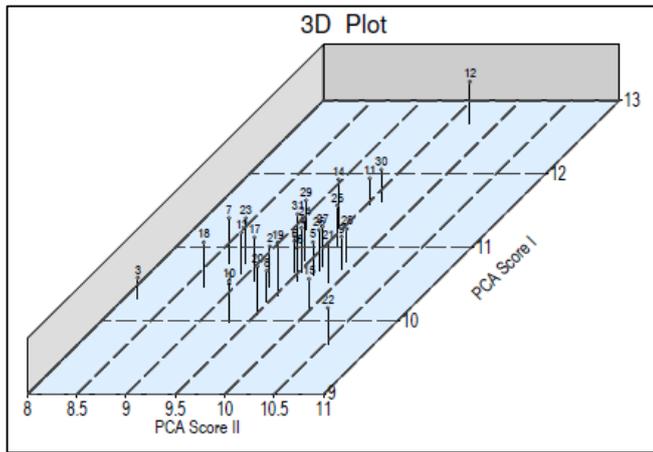


Fig 4: Patterns of relationships among 31 *Brassica* genotypes revealed by Principal Coordinate Analysis based on morphological data. For details of genotypes please refer Table 1

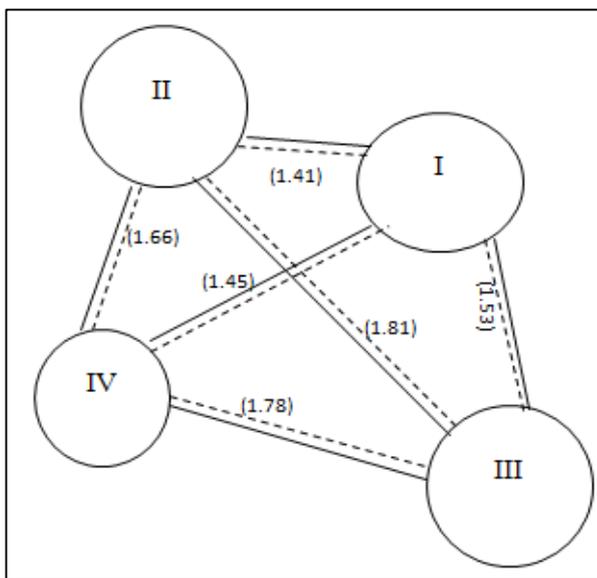


Fig 5: Cluster Diagram

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