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Evaluation of Half-sib Progeny of *Grewia optiva* Drummond under Nursery Conditions

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Abstract: Significant variability was observed for different years and between selected populations for all seedling growth characteristics in two-year-old half-sib progenies of *Grewia optiva*. Phenotypic coefficient of variation (PCV) found higher than the genotypic coefficient of variation (GCV) for all the characters. GCV and PCV values were observed at their maximum for the number of branches (GCV: 20.97% and PCV: 24.06%) and branch angle (GCV: 22.30% and 24.92%). The highest heritability was recorded for leaf area, i.e., 91.22, and highest genetic advance (64.20) for seedling height and highest genetic advance as a percent of mean (41.12%) were observed for branch angle. The root/shoot ration had the lowest values of heritability, genetic advance, and genetic advance as a percentage of mean. The principal components (PCI-PCIII) cumulatively accounted for 71.07 per cent of the total variation. PCA I explained 44.43% of the total variance. The genetic divergence divided thirty-five populations into two major clusters. The maximum inter-cluster distance was observed between population Dharkyari (SI-5) and population Gangal (MA-2).

Keywords: *Grewia optiva,* Genetic gain, Genetic divergence, Variability, Heritability

Grewia optiva is locally called Bhimal, Beul, or Dhaman belongs to the Tiliaceae family and is native to India. It is a unique medicinal tree of the sub-Himalayan terrain, often used for fibre and fodder by local farmers. The genus *Grewia* was named after Nehemiah Grew (1964-1712), the founder of plant physiology. Bhimal (*Grewia optiva*) is a medium-sized multipurpose nutritious fodder tree species growing in sub-tropical climates of the north-western Himalayas, generally raised on terrace risers and fairly well distributed up to middle elevations (500-2500 masl) in India, Pakistan, and Nepal (Semwal et al 2002). It is preferentially grown for fodder in the hills of Uttarakhand, Himachal Pradesh, and Nepal, etc., due to its high palatability, faster growth, easy propagation and high forage yield over other tree species (Mukherjee et al 2018) and its ability to retain an appreciable amount of nutrients in its leaves (Katoch et al 2017). Livestock rearing combined with agriculture is a common practise in the hilly areas of the country and it plays an important role in the economy of the country. The availability of nutritious fodder is primarily dependent on the availability of tree fodder (Roder 1992), particularly during the winter months when the availability of quantity (Khanal and Subba 2001) and quality (Vishvakarma et al 1998, Roder et al 2003) of green fodder is limited. *Grewia optiva* leaf fodder is almost as nutritious as that of leguminous crops, containing high digestibility, good vitamins and minerals, and it also improves the microbial growth and digestion of cellulosic biomass in the rumen of livestock (Singh 1982). Its leaves are fairly rich with 17.4-21.0% crude protein, 17-21.5% crude fiber, 10.4-21.5% total ash, 4.2-6.0% ether extract, and 40.4-50.2% nitrogen free extract (Sankhyan and Bhagta 2016) and do not contain tannins (Orwa et al 2009). Crude protein is highest in young leaves and in winter leaves but decreases during the rainy season. Verma et al. 2014, Orwa et al 2009).The high calorific value (4920 kcal kg^{-1}) of the tree wood makes it an excellent fuel wood and alternative energy source. It provides fibre, edible fruits and is also used as a traditional medicinal tree for treating various diseases like cough, dysentery, diarrhoea, small pox, malaria, typhoid, intestine and bladder with irritable conditions, rheumatism and eczema (Chopra et al 1956). Besides producing valuable products, the tree also provides a variety of ecological functions and associated services (Verma et al 2014). A considerable amount of organic matter is also added to the soil through the litter fall of Bhimal leaves (Kar *et al* 2019).

Progeny testing is a prerequisite to estimating the genetic worth of parents while screening the naturally available genetic variations so as to isolate good genotypes rather than merely selecting good phenotypes and to achieve maximum gain per unit area. The aim of the present study was to delineate genetically divergent best nutritive strains of *G. optiva* Drummond in different geographical regions of Himachal Pradesh and generate promising breeding material or heterotic vigour through hybridization between

genetically distant families of *G. optiva.* Hence, keeping in view the above aim, the present investigation was executed.

MATERIAL AND METHODS

The present investigation on the evaluation of half-sib progeny of *G. optiva* Drummond was carried out for two years, *i.e.*, 2019-20 and 2020-21 at College of Forestry, Dr. Y. S. Parmar University of Horticulture and Forestry, Nauni, Solan, Himachal Pradesh. Taking into account the rich genetic diversity and phenotypically superior plant populations of *G. optiva,* thirty-five populations of the 20 cm-30 cm diameter class (five populations in each district) (Table 1) were selected from seven districts of Himachal Pradesh, namely Kangra, Mandi, Bilaspur, Solan, Sirmaur, Una, and Hamirpur. To study the growth performance of the progeny under nursery conditions, seeds were collected, processed, and maintained separately on an individual tree basis. Seeds obtained from different selected trees were depulped by soaking in lukewarm water, dried for 3-4 days and sown during April–May 2019 in poly bags (in three replications) under Shilli nursery conditions. Shilli nursery is situated at 30.904486° N, 77.096733° E at an altitude of 1300 m above mean sea level, with an average annual rainfall of 1262 mm. The germination of seeds takes place in 10-15 days of sowing.

Different growth characteristics of progenies, *viz.,* seedling height, root/shoot ratio, internodal length, leaf length, and leaf breadth, were measured with the help of a scale; basal diameter with a vernier calliper, branch angle with a protractor; number of branches, number of leaves visually counted and leaf area measured with the help of a leaf area meter. The data on seedling growth characteristics was recorded for two years, and pooled data for two years is presented under the present investigation. The genotypic coefficient of variation (GCV) and phenotypic coefficient of variation (PCV) analysis (Allard 1960) and genetic advancement and heritability as per Burton (1952). Genotypic correlation coefficients and principal component analysis were calculated using OP-STAT (Sheoran *et al* 1998) and PAST (Hammer *et al* 2001). A cluster analysis was performed on PAST.

RESULTS AND DISCUSSION

The analysis of variance (Table 2) was conducted for all the progeny characteristics of thirty-five selected populations of *Grewia optiva*. Significant differences were observed for all seedling characteristics between different years and for different populations. A significant interaction was also observed between population/treatments and years.

Genetic variability parameters revealed that the phenotypic coefficient of variation (PCV) was higher than the genotypic coefficient of variation (GCV) for all the characters under observation (Table 3). A narrow difference existed between PCV and GCV in most characters, showing that they were comparatively stable to environmental pressure, which means environmental influences were very low and hence the phenotypic performance of traits can be used as a

Table 1. Details of thirty-five populations selected from seven districts of Himachal Pradesh

Population No.	Population name	Population code	District
1	Kothi kanwal	SO-1	Solan
2	Unchagaon	SO-2	Solan
3	Nerikalan	SO-3	Solan
4	Gaddo	S0-4	Solan
5	Devera	SO-5	Solan
6	Machair	SI-1	Sirmaur
7	Jajjar	SI-2	Sirmaur
8	Neharbag	SI-3	Sirmaur
9	Badon	SI-4	Sirmaur
10	Dharkyari	SI-5	Sirmaur
11	Kant	UN-1	Una
12	Navami	UN-2	Una
13	Kharunibangana	UN-3	Una
14	Thanakalan	UN-4	Una
15	Lamlehri	UN-5	Una
16	Katoi	KA-1	Kangra
17	Balugloa	KA-2	Kangra
18	Purana kangra	KA-3	Kangra
19	Dohan	KA-4	Kangra
20	Balla	KA-5	Kangra
21	Janhen	HA-1	Hamirpur
22	Jhinkari	HA-2	Hamirpur
23	Harbalneri	HA-3	Hamirpur
24	Anu khurd	HA-4	Hamirpur
25	Bhaleth	HA-5	Hamirpur
26	Patta	MA-1	Mandi
27	Gangal	MA-2	Mandi
28	Bagla	MA-3	Mandi
29	Balt	MA-4	Mandi
30	Bharnoi	MA-5	Mandi
31	Ghumarwin	BI-1	Bilaspur
32	Barthi	BI-2	Bilaspur
33	Kuthera	BI-3	Bilaspur
34	Jukhala	BI-4	Bilaspur
35	Nehari	BI-5	Bilaspur

criterion for selection. GCV and PCV were observed at their maximum for the number of branches (GCV: 20.98% and PCV: 24.07%) and branch angle (GCV: 22.31 and 24.93%). Genetic parameters permit identifying the action nature of involved genes as well as evaluating the efficiency of different selection methods and strategies (Cruz et al 2014). The highest heritability was recorded for leaf area, i.e., 91.22; the highest genetic advance (64.204) for seedling height and genetic advance as per cent of mean (41.127%) was observed for branch angle (Table 3). The root/shoot ration had the lowest values of heritability, genetic advance, and genetic advance as a percentage of mean. The relationship between heritability and genetic advance of a trait aids breeders in predicting the performance of those traits in future generations as well as their response to selection. Similar findings were reported by Kundal et al (2020) in their study on half-sib progeny evaluation of *Toona ciliata*.

The Pearson genotypic and phenotypic correlation was also observed for all the characters under study. The seedling height showed a highly significant and positive genotypic and phenotypic correlation (Table 4, Fig. 1) with basal diameter (rg: 0.702, rp: 0.540), number of branches (rg: 0.564, rp: 0.404), leaf area (rg : 0.708, rp: 0.647), leaf length (rg: 0.554, rp: 0.437), leaf breadth (rg: 0.422, rp: 0.328) and with the number of leaves (rg: 0.865, rp: 736). Root: shoot showed a strong genotypic correlation with branch angle (rg : 0.366). There was a strong genotypic correlation observed between basal diameter and the number of branches and leaf length. Basal diameter also showed a strong genotypic and phenotypic correlation with leaf area (rg: 0.569, rp: 0.467), and with the number of leaves (rg: 0.706, rp: 0.454). Strong and positive genotypic and phenotypic correlations were

Table 2. Anova for different seedling/half sib progenies growth characteristics of *Grewia optiva*

Source	DF	SH	RS	BD	NB	IL	BA	LA	LL	LB	NL
		MS	MS	MS	MS	MS	MS	MS	MS	MS	MS
Rep/year	4	0.898	0.000	0.071	0.087	0.013	0.655	1.593	0.045	0.007	8.277
Treatments	34	786.27**	0.015**	11.80**	28.78**	2.364**	540.010**	181.92**	5.707**	3.964**	859.56**
Year	1	17407.78**	0.0005	2531.0**	6.97**	44.96**	17431.07**	7324.26**	42.33**	25.36**	158148.24**
Treat* year	34	50.25**	0.004**	3.36**	3.92**	0.340**	59.68**	8.35**	0.38**	0.3203**	206.04**
Pooled error	136	2.346	0.00	0.058	0.064	0.007	1.109	1.721	0.036	0.014	6.732
Total	209										
CV		2.583	2.742	2.757	2.603	2.521	2.625	2.686	2.595	2.713	3.033
CD 5% Y		0.418	NS	0.066	0.069	0.023	0.287	0.358	0.052	0.032	0.708
CD 5% T		8.318	0.080	2.152	2.326	0.684	9.065	3.391	0.729	0.664	16.842
CD 5% Y*T		2.473	0.021	0.389	0.408	0.138	1.700	2.118	0.305	0.192	4.189

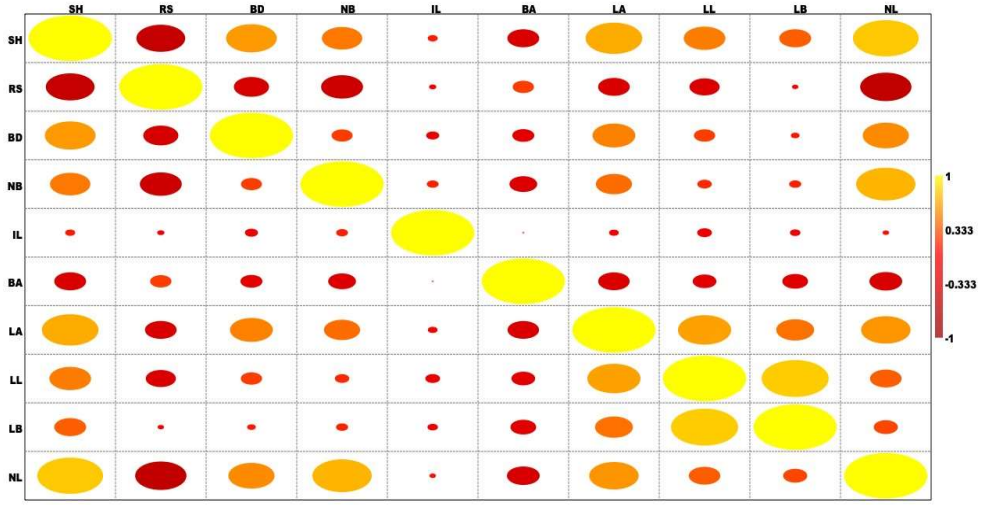
Significant at 5 % level, Where; SH-seedling height, R/S-root/shoot ratio, BD-basal diameter, NB- number of branches, IL-internodal length, BA- branch angle, LA-leaf area, LL- leaf length, LB- leaf breadth, NL- number of leaves, CV-coefficient of variation, CD-critical difference, Y-year, T-Treatment

Table 3. Genetic estimates for growth characteristics of *Grewia optiva* progenies

Parameters	Range	Coefficient of variability		H^2 (Heritability)	GA (Genetic advance)	GAM (Genetic advance as percent of mean)
		GCV	PCV			
SH (cm)	30.99-87.44	18.674	19.908	87.985	64.204	36.084
R/S	0.4-0.61	8.907	12.185	53.426	0.191	13.411
BD (mm)	3.78-13.99	13.578	18.201	55.655	5.468	20.867
NB	5.61-13.67	20.978	24.066	75.983	10.965	37.669
IL (cm)	1.68-5.14	17.157	19.831	74.846	3.105	30.577
BA (°)	15.54-66.66	22.308	24.927	80.094	49.486	41.127
LA (cm^2)	31.45-62.45	11.014	11.532	91.22	31.746	21.671
LL (cm)	5.3-8.99	12.925	13.832	87.319	5.438	24.88
LB (cm)	3.02-5.87	17.79	19.291	85.047	4.441	33.797
NL	45.13-137.21	12.202	15.581	61.328	50.509	19.684

See Table 2 for details. GCV-genotypic coefficient of variability, PCV-phenotypic coefficient of variability

recorded between the number of branches and leaf area, i.e., rg: 0.459, rp: 0.396, and between the number of branches and number of leaves per seedling. Non-significant and negative correlations were observed between internodal length and all other observed characteristics. There was a negative and significant genotypic and phenotypic correlation observed between branch angle and all other characteristics. Leaf area showed a highly significant correlation with leaf length (rg: 0.688, rp: 0.585), leaf breadth (rg: 0.496, rp: 0.399) and with the number of leaves per seedling (rg: 0.663, rp: 0.536). Strong phenotypic and genotypic correlations were observed between leaf length



See Table 2 for details

Fig. 1. Presentation of phenotypic and genotypic correlation between different seedling growth characteristics

Table 4. Genotypic and phenotypic correlation among growth characters of *Grewia optiva* progenies

		SH	RS	BD	NB	IL	BA	LA	LL	LB	NL
SH	rg	1.000									
	rp	1.000									
RS	rg	-0.669**	1.000								
	rp	-0.533**	1.000								
BD	rg	0.702**	-0.545**	1.000							
	rp	0.540**	-0.344**	1.000							
NB	rg	0.564**	-0.603**	0.317**	1.000						
	rp	0.404**	-0.424**	0.188^{NS}	1.000						
IL	rg	0.133^{NS}	-0.057^{NS}	-0.147^{NS}	0.135^{NS}	1.000					
	rp	0.083^{NS}	-0.073^{NS}	-0.139^{NS}	0.119^{NS}	1.000					
BA	rg	-0.415**	0.366**	-0.277*	-0.369**	-0.049^{NS}	1.000				
	rp	-0.335**	0.176^{NS}	-0.235*	-0.284*	0.050^{NS}	1.000				
LA	rg	0.708**	-0.457**	0.569**	0.459**	-0.098^{NS}	-0.373**	1.000			
	rp	0.647**	-0.319**	0.467**	0.396**	-0.100^{NS}	-0.363**	1.000			
LL	rg	0.554**	-0.498**	0.335**	0.154^{NS}	-0.170^{NS}	-0.312**	0.688**	1.000		
	rp	0.437**	-0.255*	0.178^{NS}	0.162^{NS}	-0.153^{NS}	-0.239*	0.585**	1.000		
LB	rg	0.422**	-0.083^{NS}	0.145^{NS}	0.131^{NS}	-0.121^{NS}	-0.339**	0.496**	0.833**	1.000	
	rp	0.328**	-0.042^{NS}	0.047^{NS}	0.126^{NS}	-0.101^{NS}	-0.262*	0.399**	0.775**	1.000	
NL	rg	0.865**	-0.689**	0.706**	0.951**	0.148^{NS}	-0.418**	0.663**	0.491**	0.356**	1.000
	rp	0.736**	-0.576**	0.454**	0.545**	0.003^{NS}	-0.360**	0.536**	0.280*	0.221^{NS}	1.000

See Table 2 for details

and leaf breadth (rg: 0.833, rp: 0.775) and the number of leaves (rg: 0.491, rp: 0.280). These results find support with the findings of Deepanjli (2018) in different seed sources of *Toona ciliata,* Thakur and Thakur (2015) in *Melia azedarach*, Singh et al (2015) in *Populus deltoides.*

PCA (principal component analysis) results showed that the principal components I (PC I), principal component II (PC II) and principal component (PC III) gave eigenvalues >1.0. PC I accounted for 44.43% of the total variation (Table 5). PC II accounted for 16.088 % of total variation and PC III accounted for 10.553 % of total variation. PC I was positively associated with the characteristics viz., seedling height, basal diameter, number of branches, leaf area, leaf length, leaf breadth, and number of leaves. The PC II was strongly associated with the characteristics, *viz.,* number of branches and internodal length. PC III is associated positively with internodal length, leaf length, and leaf breadth. Thus, the use of these characteristics will help in saving a considerable amount of time for the identification and selection of the best genotypes of *Grewia optiva*. The principal component analysis (PCA) is one of the powerful statistical methods widely applied to classify phenotypic traits in tree germplasm into groups based on similarities. PCA guides the choice of parents for genetic improvement (Afuape et al 2011, Beheshtizadeh et al 2013). PCA reduce the original variables into a new set of uncorrelated variables known as principal components (PCs). These PCs clarify the connections between traits and divide the total variance of original traits into a small number of uncorrelated new variables (Wiley and Lieberman 2011).

A biplot (Fig. 2) was also drawn using the values of PCA I and PCA II. The PCA allows visual differentiation among entries and identify possible associations by providing a two-dimensional scatter plot consisting of individual entries. The geometric distance among individuals in this plot reveals the genetic distance among them. Amalgamation of individuals in a similar quadrant of plot may indicate a group of genetically related individuals. (Warburton et al 2002). The higher the coefficients of particular characters, more it is related to the respective principal component axis. Four grouping of seedling growth characteristic was observed in Biplot and some overlapping occurred within groups demonstrating the relatedness of the seedling growth characteristic of different seed sources.

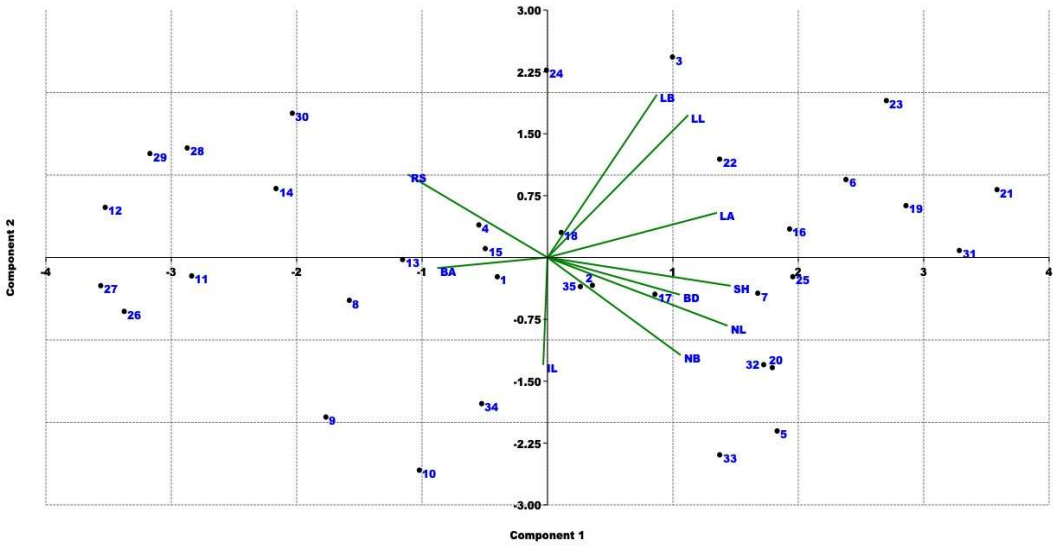
In the biplot graph of PCA, the quadrant I (+, +) consisting of 9 different populations formed the cluster 1, which was highly influenced by three growth characteristics, viz., leaf breadth, leaf length, and leaf area. The cluster II, corresponding to the quadrant II (-, +), contained 9 different populations and was highly influenced by seedling growth characteristics, viz., seedling height, basal diameter, number of leaves, and number of branches. Similarly, the cluster III corresponding to quadrant III (-, -) consisted of nine different populations and was influenced by two growth characteristics, i.e., internodal length and branch angle. The cluster IV corresponding to quadrant IV (-, -) consisted of eight different populations and was influenced by one growth characteristic, i.e., root: shoot ration. The quadrants III and IV are least influenced by the seedling growth characteristics under study.

Genetic divergence analysis divided thirty-five populations into two major clusters and three sub-clusters based on different seedling growth characteristics under consideration and presented through a dendrogram (Fig. 3) using Ward's method. Cluster I and Cluster II comprised of 23

Table 5. Principal component analysis of different growth characteristics of *Grewia optiva* progenies

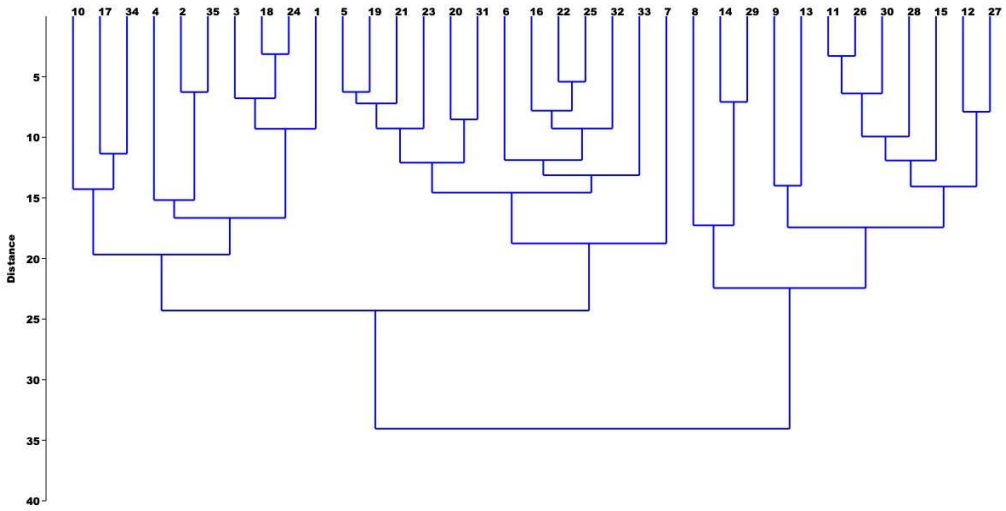
Parameters	PC 1	PC 2	PC 3	PC 4	PC 5	PC 6	PC 7	PC 8	PC 9	PC 10
SH	0.417	0.098	-0.005	0.187	-0.215	0.05	0.156	0.625	-0.533	0.186
RS	-0.318	-0.287	0.064	-0.206	-0.288	0.747	0.138	0.078	0.055	0.317
BD	0.301	0.129	-0.527	0.122	-0.487	0.072	0.239	-0.546	-0.049	0.032
NB	0.303	0.338	0.181	-0.234	0.54	0.368	0.012	-0.368	-0.331	0.186
IL	-0.01	0.371	0.731	0.286	-0.442	0.009	-0.067	-0.2	0.081	0.006
BA	-0.251	0.037	-0.155	0.844	0.327	0.294	0.058	-0.019	-0.019	-0.05
LA	0.385	-0.154	-0.073	0.062	-0.103	0.352	-0.758	0.04	0.085	-0.317
LL	0.32	-0.492	0.143	0.23	0.097	-0.222	-0.118	-0.17	0.178	0.67
LB	0.249	-0.563	0.319	0.047	0.051	0.066	0.432	-0.158	-0.151	-0.526
NL	0.409	0.236	-0.007	-0.01	0.134	0.186	0.34	0.273	0.73	-0.04
Eigenvalue	4.443	1.609	1.055	0.800	0.697	0.545	0.362	0.258	0.138	0.093
% variance	44.433	16.088	10.553	8.004	6.974	5.450	3.616	2.578	1.378	0.928
Cumulative variance	44.433	60.521	71.074	79.078	86.051	91.501	95.117	97.695	99.072	100.000

See Table 2 for details



Where, 1-Kothi kanwal population (SO-1), 2- Unchagaon population (SO-2), 3- Nerikalan (SO-3), 4- Gaddo (SO-4), 5-Devwra- (SO-5), 6-Machair (SI-1), 7-Jajjar(SI-2), 8-Neherbhag (SI-3), 9- Badon (SI-4), 10-Dharkyari (SI-5), 11- Kant (UN-1), 12- Navami (UN-2), 13-Kharunibangana (UN-3), 14- Thanakalan (UN-4), 15-Lamlehri (UN-5), 16- Katoi, (KA-1), 17-Balugloa (KA-2), 18- Purana kangra (KA-3), 19- Dohan (KA-4), 20-Balla (KA-5), 21- Janhen (HA-1), 22- Jhinkari (HA-2), 23-Harbalneri (HA-3), 24-Anu khurd (HA-4), 25- Bhaleth (HA-5), 26- Patta (MA-1), 27- Gangal (MA-2), 28- Bagla (MA-3), 29- Balt (MA-4), 30-Bharnoi (MA-5), 31- Ghumarwin (BI-1), 32- Barthi (BI-2), 33- Kuthera (BI-3), 34- Jukhala (BI-4), 35- Nehari (BI-5)

Fig. 2. Biplot between principal component 1 and 2



See Fig 2 for details, SH-seedling height, R/S-root/shoot ratio, BD-basal diameter, NB- number of branches, IL-internodal length, BA- branch angle, LA- leaf area, LL- leaf length, LB- leaf breadth, NL- number of leaves

Fig. 3. Dendrogram depicting genetic divergence of thirty – five populations of Grewia optiva

and 12 different populations, respectively. The maximum inter-cluster distance was observed between population no. 10 (Dharkyari, Sirmaur District) and population no. 27 (Gangal, Mandi District). Whereas, the maximum intra-cluster distance was observed between population no. 7 (Jajjar, Sirmaur District) and population no. 10 (Dharkyari, Sirmaur District) in cluster I and population no. 8 (Neharbag, Sirmaur District) and population no. 27 (Gangal, Mandi district) in cluster II. In a study conducted by Navya et al (2021) in sorghum (*Sorghum bicolor* L) reported that 20 genotypes were classified into four distinct clusters. The maximum (6) and lowest (4) number of genotypes were in clusters I and IV respectively. Clusters II and IV (150.99) and cluster III and IV (150.99) had the greatest and smallest inter

cluster. Therefore, hybridization between the progenies of distant populations may produce more hybrid vigour. Similar results were reported by Sehgal et al (1995) in chir pine, Behera et al (2017) in *Eucalyptus,* Kumar et al (2016) in *Dalbergia sissoo,* Kundal et al (2020) in Toona ciliata and Mohanraj et al (2022) in *Toona ciliata.*

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