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GENETIC DIVERSITY BASED ON MORPHOLOGICAL TRAITS IN WALNUT (*JUGLANS REGIA* L.) LANDRACES FROM KARAKORAM REGION-I

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Abstract

Walnut is one of the most important nutritive nut crops and widely grown in Gilgit-Baltistan region of Pakistan. In the present study 19 local landraces were analyzed for morphological traits to investigate genetic diversity and identify promising landraces for cultivar development. Multivariate analyses showed high variation for morphological traits and nut and kernel characteristics. Cluster analyses depicted diversity among the local land races which separated them into 2 major clusters groups, showing more association to morphological differences. PCA revealed that the 1st four principal components (PC's) possessed Eigen value >1.0, where PC1 and PC2 contributed total variance of 41.65% and 23.42% respectively with total variance (65.05%) showing maximum factor loadings by kernel ratio, shell%, kernel yield and nut width by the first two PC's. Pearson correlation coefficient among walnut landraces revealed positively significant correlation between shell yield and nut weight(r=0.96), kernel yield and nut width(r=0.85), whereas negative correlation were observed (r = -0.89 and r = -0.76) between kernel ratio with shell yield and nut weight respectively. A wide range of diversity was observed among the local landraces from Karakoram regions and the landrace HKK and GNAG were reported as promising one with highest kernel ratio. These landraces are potential for future breeding of nut crops with distinct morphological traits.

Key words: Genetic diversity, Landraces, walnut, Gilgit-Baltistan, Morphology, PCA.

Introduction

English or Persian walnut (*Juglans regia* L.) is a nut crop belongs to family Juglandaceae. The genus *Juglans* contains 20 species producing edible nuts and characterized by a monoecious and heterodichogamous habit (Gleeson, 1982). In Pakistan it is known as Akhrot (Urdu), while locally it is known as Khakai (Shina) and Starga (Balti). It is distributed over a wide range of geographical conditions, including southern Europe, eastern Asia, and the USA (Krussman, 1986), however it grows well in temperate climatic conditions (Arzani *et al.,* 2008; Cosmulescu & Botu, 2012). It is native of a region stretching from the Balkans eastward to the Western Himalayan chain (Fernandez-Lopez *et al.,* 2002). Islamic Republic of Iran is considered as the center of origin and diversity of walnut (Arzani *et al.,* 2008). The global average walnut production in Asian regions was reported 68.4% during the last five years (2008-2012), followed by America (19.1%). The leading walnut producing countries are Republic of China, Islamic Republic of Iran, USA and Turkey (Anon., 2013). Walnut production in Pakistan was reduced during the last many years. However, its production during the last two years was 11.5 thousand tones with increasing trend (Anon., 2013).

Genetic variability in walnut populations has been observed very high and exists in various parts of the world (Sharma & Sharma, 2001; Díaz & Fernández-López, 2005; Khan *et al.,* 2010). Morphological characters are considered to be an option for selection and classification of promising germplasm. Morphological variation in nut sizes, thickness of shell, kernel percentage and yield of kernels in walnut trees from various geographical areas have been reported (Casal *et al.,* 2005; Zeneli *et al.,* 2005; Karimi *et al.,* 2014). Statistical methods like PCA, cluster analysis and correlation can be used as functional tools for screening promising landraces and can be useful in breeding program (Mohammadi & Prasanna, 2003; Shinwari *et al.,* 2013; Zada *et al.,* 2013). Promising walnut genotypes were identified from various regions (Ozkan & Koyuncu, 2005; Arzani *et al.,* 2008; Cosmulescu & Botu, 2012).

Being floristically rich area, Gilgit-Baltistan (G.B) and adjoining mountain areas of Pakistan host majority of its endemic flora (Ali & Qaiser, 1986), medicinal plants with unique traditional uses (Shinwari & Gilani, 2003; Hussain *et al.,* 2011) and variety of fruit cultivars (Virk *et al.,* 2003). The study area is situated at a very important geographic place bordering Khyber Pakhtunkhwa (KPK) China, India and the territories of A. J & Kashmir. These areas are well known for rich biodiversity as they are situated at the junction point of three great mountain ranges (Shinwari *et al.,* 2000). The K-2 *Chogho-Ree* (8611 m), Gashabrum-I (8068 m), Gashabrum II (8035m), Broad Peak (8047 m), Mashabrum (7821 m) and other mountain peaks with more than 7000 m are the famous peaks situated in G.B (Hussain *et al.,* 2011). The study area has unique climate with least influence from easterly monsoon and major precipitation received through westerly winds (Khan, 1996). The temperature is very warm during summer and cold in winters. The annual mean temperature is comparatively low in Hunza 11.2°C as compare to Gilgit 15.9°C (Table 2). The average annual rainfall in Hunza was reported 137 mm/ year than that of Gilgit region 141 mm/ year (Eberhardt, 2004). However, the mean maximum rainfall is greater in summer season 23.6 mm than that of winter season 2.26 mm (Khan *et al.,* 2011). Such kind of climatic conditions fits for walnut production and can tolerate extreme temperature (-11° C to <38°C) for optimum plant growth (Serr, 1969). The wild seedlings of walnut have been reported in vast area from Eastern Europe, across Turkey, Iran and Afghanistan to northern mountains of Himalayas (Serr, 1969; Woodroof, 1979).

In Gilgit-Baltistan, it also grows wildly by seed dispersal mechanism since ancient times and concept of grafting and budding is very scarce. Since, G.B is famous for walnut production, trees originated from seed have wide distribution across the region and total area under walnut is 1429 thousand Ha. Both Gilgit and Hunza-Nagar Districts contribute 30.16% of total walnut production (6577 metric tons) in G.B (Anon., 2009). Information regarding local walnut landraces and its documentation from different valleys of G. B has not been done and scientific knowledge regarding walnut from Karakoram Mountains of Pakistan is lacking so far. Hence, the aim of this research was to evaluate and identify promising landrace within the walnut population from the study area of Gilgit and Hunza-Nagar Districts. In this context this is the very first attempt in the series of walnut documentation from Karakoram mountain regions of G.B, so that these landraces may be use as breeding material in future cultivar development programs.

Materials and Methods

The present study was carried out during September to November, 2013 and nut samples were collected from different areas of Hunza-Nagar and Gilgit Districts, Gilgit-Baltistan (Fig. 1).

Sampling: Total 19 local landraces of walnut were collected for the study covering different villages of the study area from farmers' field during the peak harvesting season thereby assembling into different groups with distinct morphological features. The allocated code names and location were noted during the field study (Table.1). All collected samples were washed by removing exocarp and dried as per standard practice to get stabilized production weight and preventing deterioration to obtain healthy nut samples (Olson & Coates, 1985).

Table 1. Sampling site and codes of walnut landraces of District Hunza-Nagar and Gilgit.

S. No.	Codes	Sampling location
1.	HKH 1	Karimabad
2.	HKG	Karimabad
3.	HKK	Karimabad
4.	HKH 2	Karimabad
5.	HKO	Karimabad
S.	HKQ 1	Karimabad
7.	HKQ 2	Karimabad
8.	HK 1	Karimabad
9.	HK 2	Karimabad
10.	GDH	Danyore
11.	GDA	Danyore
12.	GNAG	Nagaral Gilgit
13.	GKH	Khomer
14.	HKB 1	Karimabad
15.	HAL 2	Altit
16.	HK 3	Karimabad
17.	HAL 3	Altit
18.	HAL 7	Altit
19.	HAL 8	Altit

Fig. 1. Study area (sampling area highlighted in circle) Gilgit-Baltistan, Source: (Map data, 2015 Google).

Morphological parameters: The independent morphological traits of walnut in the study area were evaluated for quantitative traits with varying sizes for 30 randomly chosen nut samples by using vernier caliper (A-015-145) and electronic balance (accuracy of 0.01g). The morphological traits of walnut landraces were recorded for Nut length(cm), Nut width(cm), Nut weight(g), Shell thickness(mm), kernel length (cm), kernel width (cm), Kernel yield (g) as weight of kernel obtained after removing the shell, Shell yield expressed in grams after separating kernel from the nut, Shell (%) as (shell yield to nut weight ×100) and kernel ratio (kernel weight to nut weight ratio ×100) for each landraces were evaluated according to the walnut international descriptor (Anon., 1994).

Statistical analysis: The data were computed for descriptive statistics, cluster analysis and correlation by using statistical software packages i.e., STATISTICA (StatSoft Inc. 5.5), Statistix 8.1 (USA) and MS Excel program 2007. Data were analyzed statistically using multivariate analysis such as Principal Components Analysis (PCA) for determining genetic diversity based on morphological parameters. Statistically significant principal components (PCs) were selected according to Eigen significant criteria for factor analysis (Kaiser, 1960). To find out the relationship among the nut and kernel traits, the Pearson correlation coefficient was performed (Steel & Torrie, 1980). To assess genetic relationship among landraces, the data matrix was analyzed for similarity coefficient according to Nei and Li (1979), using unweighted pair-group method with arithmetic average (UPGMA) as clustering method.

Results

The results showed that evaluated landraces had high variation for morphological traits and distinct characters (Fig. 2). The study showed high variability for nut and kernel characteristics, the summarized results with descriptive statistics for 10 quantitative characters of walnut are presented in Table 3. The highest coefficient of variability (56.04) was observed in NL, with standard deviation (2.79). Mean KY was in a range of 3.37-5.68 g (4.65±0.72 g) among landraces, whereas SHY was recorded in a range of 3.30-13.18 (5.19±2.15). KR in local landraces was recorded in a range from 27.28-56.88% (48.39±6.35). NWT was recorded in bit broader range having 6.93-18.12 g with mean value 9.85±2.51 and SHT was observed as mean value 1.18±0.05 mm (Table 3). Out of all landraces highest kernel ratio was recorded by HKK (56.88%) followed by GNAG (55.03%), HK3 (53.74%) and HK2 (53.16%). However, only two landraces GDA and GKH were reported to have lowest kernel ratio as 27.28% and 41.66% respectively.

According to the cluster analysis, there was diversity among local landraces, which separated 19 landraces of walnut into 2 major clusters groups (Fig. 3). These landraces were characterized by different morphological characters. The members of cluster 1 were sub-divided into two groups, where majority of them have linkage distance less than 20. The highest linkage distance was developed between landrace GDA vs HKK, whereas it was minimum between GDH vs HKQ2. Local landrace (GDA) was placed separately in the last group, indicating that it showed the greatest morphological difference from the others. Correlation of coefficient between different traits of walnut genotype included in the study revealed significant correlations among different variables such as nut weight and nut width (r=0.75), kernel width and nut width (r=0.64), kernel yield and nut width (r=0.85), shell yield and nut width (r=0.60), kernel yield and nut weight (r=0.60), shell yielding and nut weight (r=0.96), shell% and nut weight(r=0.76), kernel ratio and nut weight(r=-0.76), kernel yield and kernel width(r=0.51), shell% and shell yield(r=0.89), a negative correlation with significant differences were also observed (r = -0.89 and r = -0.76) between kernel ratio with shell yield and nut weight respectively. The highest and significant positive correlation was found between shell yielding and nut weight(r=0.96) (Table 4).

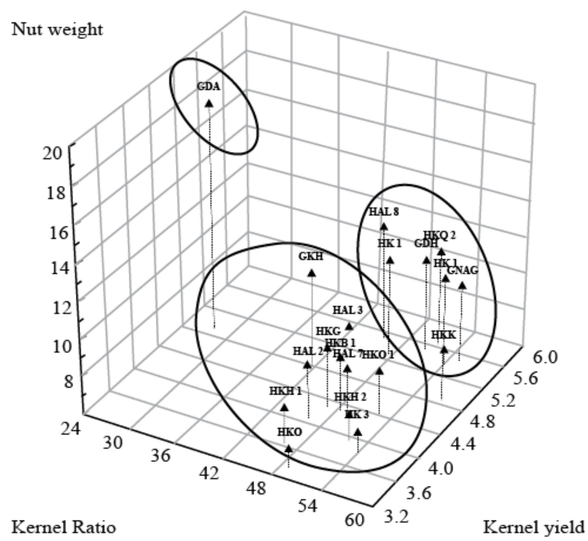

Fig. 2. A 3-D view for Genetic diversity among local walnut landraces based on yield traits from Hunza-Nagar and Gilgit.

Principal component analysis (PCA) of quantitative traits in local walnut landraces revealed maximum variation and the 1st four PC's showed Eigenvalues > 1.0 where PC1 possessed the highest Eigenvalue (4.16). Out of four major PC's the first PC showed significant factor loadings for morphological traits viz. kernel ratio, shell%, shell yield and nut weight. Whereas, significant factor loadings for kernel yield, nut width and kernel width by PC2, shell thickness and nut length by PC3 and only kernel length showed significant factor loadings by the PC4 (Table 5). The PCA for walnut landraces revealed maximum variation by the first two PC's contributing 65.05% variance and the contribution of walnut landraces showed divergence among local landraces. The factor loadings for landrace was maximum by HAL8 (0.95) by PC1 whereas landrace HAL7 showed significant factor loadings (0.70) by PC2 (Fig. 4).

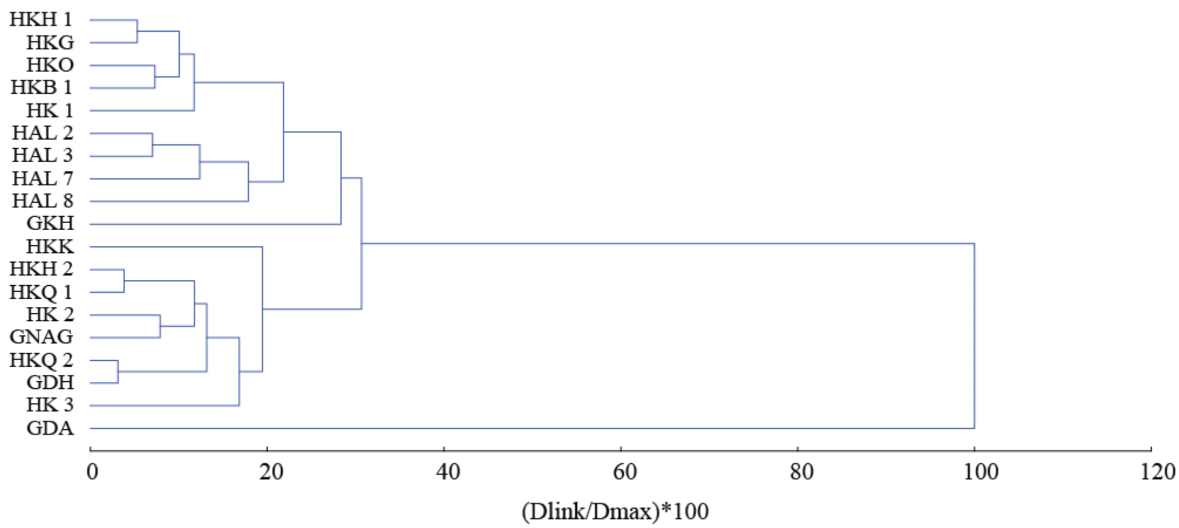

Fig. 3. Classification of local walnut landraces (19) using cluster analysis (UPGMA) based on morphological traits from Hunza and Gilgit during 2013.

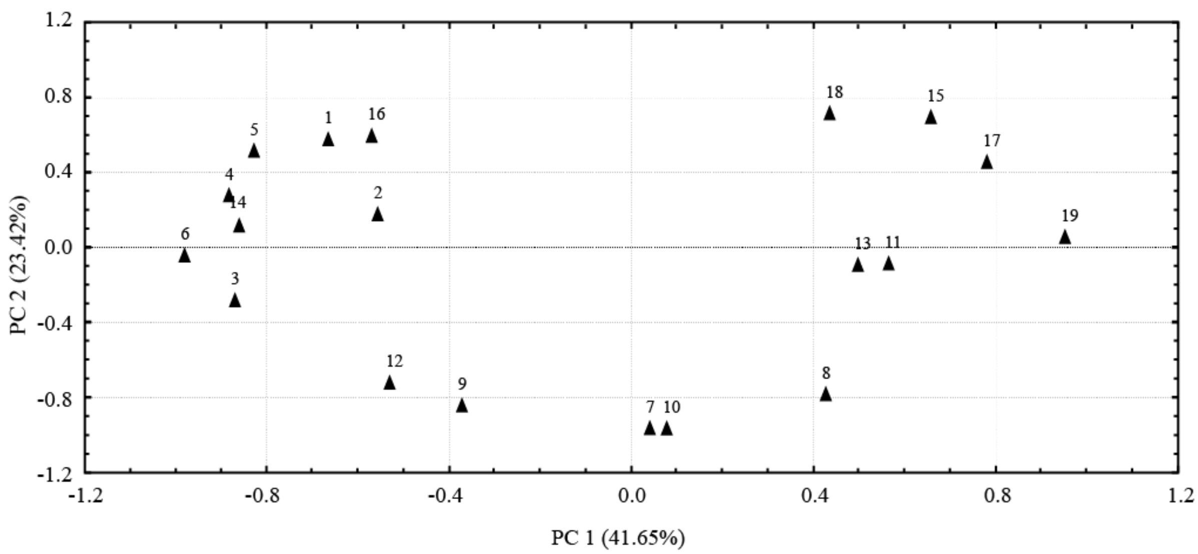

Fig. 4. Contribution of walnut landraces for the 1st two PC's by principal components analysis from the study area (serial number in the graph corresponds to walnut landraces mentioned in Table 1).

Table 2. Physical features and climate data of study area Gilgit and Hunza-Nagar.

		Study area	
		Gilgit district	**Hunza-Nagar district**
Physical features	Cropping Zone	Double cropping	Single Cropping
	Local language	Sheena	Sheena and Broshiski
	Sampling Villages	Danyor, Amphari, Khomar, Nagral	Karimabad, Olobasi, Harmaling, Baltit, Qarkat, Altit, Chikshal and Kan
	GPS Coordinate	35°55'N, 74°20'E	36°18'N, 74°40'E
	Altitude	1460 m	2300 m
Climate	Mean annual temperature	15.9° C	11.2° C
	Maximum temperature	45.0 ° C	37.8 ° C
	Minimum temperature	-9.4 ° C	-12.0 ° C
	Mean annual precipitation total	141 mm	137 mm

Table 3. Descriptive statistics for morphological traits of 19 walnut landraces from Hunza and Gilgit during 2013.

Traits	Mean ± SD	Minimum	Maximum	C.V%
NL (cm)	4.97 ± 2.79	3.24	12.13	56.04
NWI (cm)	3.07 ± 0.25	2.57	3.49	8.27
NWT (g)	9.85 ± 2.51	6.93	18.12	25.45
SHT (mm)	1.18 ± 0.05	1.10	3.0	29.32
KL (cm)	2.59 ± 0.15	2.31	2.84	5.87
KWI (cm)	2.10 ± 0.17	1.67	2.34	8.21
KY (g)	4.65 ± 0.72	3.37	5.68	15.42
SHY (g)	5.19 ± 2.15	3.30	13.18	41.48
SH %	51.61 ± 6.40	43.12	72.72	12.31
KR	48.39 ± 6.35	27.28	56.88	13.13

Nut Length(NL), Nut Width(NWI), Nut Weight(NWT), Shell Thickness(STH), Kernel Length(KL), Kernel Width(KWI), Kernel Yield(KY), Shell Yield (SHY), Shell % (SH%), Kernel Ratio(KR), Standard Deviation (SD), Coefficient of Variance (CV%)

Table 4. Correlation coefficients among nut and kernel characteristics of 19 walnut landraces of Hunza and Gilgit during 2013.

	NL	NWI	NWT	SHT	KL	KWI	KY	SHY	SH_%	KR
NL (cm)	1									
NWI (cm)	0.02	1.00								
NWT (g)	0.01	**0.75**	1.00							
SHT (mm)	0.32	-0.10	-0.07	1.00						
KL (cm)	-0.02	0.19	0.19	0.18	1.00					
KWI (cm)	0.24	**0.64**	0.17	0.10	-0.06	1.00				
KY (g)	-0.02	**0.85**	**0.60**	-0.31	0.12	**0.51**	1.00			
SHY (g)	0.02	**0.60**	**0.96**	0.02	0.18	0.03	0.36	1.00		
SH %	0.09	0.25	**0.76**	0.19	0.10	-0.22	-0.03	**0.89**	1.00	
KR	-0.09	-0.25	**-0.76**	-0.19	-0.10	0.22	0.03	**-0.89**	-1.00	1.00

Marked correlations are significant at $p<050$

Nut Length cm (NL), Nut Width cm (NWI), Nut Weight g (NWT), Shell Thickness mm (STH), Kernel Length cm (KL), Kernel Width cm (KWI), Kernel Yield g (KY), Shell Yield g (SHY), Shell % (SH %), Kernel Ratio (KR)

Table 5. Principal components for quantitative traits in walnut landraces.

	PC 1	PC 2	PC 3	PC 4
Eigenvalue	4.16	2.34	1.46	1.04
Cumulative Eigenvalue	4.16	6.51	7.96	9.00
Total Variance%	41.65	23.42	14.58	10.39
Cumulative variance %	41.65	65.06	79.64	90.03
Factor loadings				
NL	0.005	0.110	**0.771**	-0.18
NWI	0.368	**0.904**	-0.029	0.13
NWT	**0.855**	0.498	-0.091	0.09
SHT	0.110	-0.186	**0.818**	0.31
KL	0.192	0.111	0.146	**0.96**
KWI	-0.213	**0.807**	0.332	-0.10
KY	0.125	**0.905**	-0.226	0.06
SHY	**0.952**	0.279	-0.030	0.09
SH%	**0.975**	-0.114	0.109	0.02
KR	**-0.975**	0.114	-0.109	-0.02

Nut Length cm (NL), Nut Width cm (NWI), Nut Weight g (NWT), Shell Thickness mm (STH), Kernel Length cm (KL), Kernel Width cm (KWI), Kernel Yield g (KY), Shell Yield g (SHY), Shell % (SH%), Kernel Ratio (KR), (Factor loadings > 0.70 are significant after principal components extraction

Discussion

Walnut displays differentiation in morphology, particularly in nut characteristics. Morphological traits showed distinct variation among local landraces of walnut from Gilgit-Baltistan in the present study based on morphological traits, by PCA and cluster analysis and exhibited diversity among local landraces in their appearance. Variation in nuts and kernels related characteristic among walnut landraces of Hnza and Gilgit region showed very broad diversity. Considerable variation in *J. regia* has been reported by many researchers (Arzani *et al.,* 2008; Mosivand *et al.,* 2013). Mean Nut weight was highest in HAL8 (12.13±0.37) and Kernel weight was highest in HKQ2 (5.68g) out of all landraces. We have found much variation in nut weight between ranges of 6.93- 18.12±0.57 g. These are in agreement with the findings reported by Karimi *et al.* (2014). Shell thickness is important for protection of kernel from external effects. The thickness across all landraces was found in a range of 1.10 to 3.0 mm. Nut length and width reported were similar than that of previously reported (Karadag & Akca, 2013). There is considerable variability in nut and kernel morphological characters as they originated from seed and this ability is attributed due to seed based propagation method, high heterozygosis and dichogamy (Aslantas, 2006). Local trees with high variation showed important breeding materials and supports the findings of Zeneli *et al*. (2005) and Aslantas (2006).

Correlations among phenotypic traits may reflect underlying biological processes that are of considerable interest in genetic diversity. The significant positive correlation among nut and kernel width, kernel weight and shell yield are in agreement with Sharma and Sharma (2001), Bayazit (2012). Whereas the negative correlation between kernel ratio and shell weight also supports the findings with Bayazit (2012) as well as some other traits like shell percentage with kernel morphological attributes (Amiri *et al.,* 2010). Divergence studies using multivariate methods based on morphological characters in various species concluded that clustering method can be used to find out relationship between populations of diverse origin (Morris, 2010; Akbar *et al.,* 2011; Sultan *et al.,* 2012). In the present work, diversity analysis in local walnut landraces by clustering method showed much association based on morphological attributes, however some of the landraces in sub-cluster groups showed close linkage among them based on geographic location as well and supporting the results mentioned by Morris (2010). Based on the current results, an extensive range of genetic diversity has been explored and revealed wide morphological variation in walnut landraces of Karakoram regions. Eight (08) landraces out of 19 had kernel ratio greater than 50% and two of them (HKK and GNAG) recorded highest kernel ratio 56.88% and 55.03% respectively. These local landraces with diverse morphological traits contributing to agro-biodiversity possess potential in terms of quantitative characters and are considered as important gene pool with distinct features for cultivar development. Further studies in collaborative approach may harness agro-biodiversity in mountain areas of Pakistan. There is still need of further documentation and effective management of local genetic resources for conservation and improvement from all regions of Gilgit-Baltistan.

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