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APPLICATION OF LEAF EPIDERMAL ANATOMICAL TECHNIQUE FOR IDENTIFICATION OF SOME GRASS SPECIES FROM DISTRICT BHIMBER OF AZAD JAMMU AND KASHMIR, PAKISTAN

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Abstract

Grasses are main source of food (cereals), fodder of livestock and good source of therapeutics and cosmetics in traditional medicine systems of the world. It is rather difficult to identify and classify grasses properly. As these plants are ethnobotanically important and have been used for treatment of different disease in traditional cultural therapeutics for dyspepsia, burning sensation, piles, sexual weakness, gynecological troubles, respiratory troubles, Kidney stones, acid reflex, blood impurity, debility, diarrhea, lecuoderma, allergy, anemia, anuria, anticancer and antidiabetic. It is pertinent that identifying grass species is very difficutl task if based on only morphological features. The key purpose of this research was to identify the selected taxa of grasses by using morphometric analysis and leaf epidermal anatomical approach from District Bhimber of Azad Jammu and Kashmir (AJK). Following species grasses: *Cynodon dactylon* L., *Saccharum spontanium* L., *Saccharum ravennae* L., *Poa annua* L. and *Poa nemoralis* L. were selected for this study. In morphometric analysis; cluster was formed which divided relevant species into distinct groups (taxon). Genetic distance (GD) was calculated to explore inter and intra-species variation. It was found that taxa from Bhimber area had highest GD (135) while species from Barnala showed least GD (83). From Leaf epidermal anatomy (LEA) study, stomatal index (SI) was calculated and among *Saccharum* genus: *Saccharum spontanium* showed highest SI (23.6) while *Saccharum revennae* had SI value of 12.6. In other genus Poa: *Poa nemoralis* depicted SI of (20.4) and while *Poa annua* had SI of 12.5. Other genus Cynodon (out group): *Cynodon dactylon* showed stomatal index of (15.0). Taxonmic keys has been devised for proper identification of all taxa under this study.

Key words: Ethnobotanical grasses, Bhimber, Azad Kashmir, Taxonomy, Morphometry, Stomatal Index.

Introduction

Plants are very important for man and among these grasses have been used for multi-purporses such as food (cereals), fodder, fuel, thatching and medicines. Poaceae or the grass family is innate homogenous group of plants, containing 10,000 species, 660 genera and 50 tribes (Clayton, 1986, Lowe, 1982, Zereen *et al*., 2013). Poaceae contains 158 genera and 492 species in Pakistan (Cope, 1982). Grasses are the most broad-based of all plants. The species are more abundant in the tropics but in the temperate regions they are also rich. Adaptability of diverse species enabled them to bloom under the most assorted conditions; some are aquatic and others are extremely arid and desert place feature (Kellogg, 1998; Zereen *et al*., 2013).

Grasses reside in the earth in abundance than other analogous faction, some plants are present in hot, moist and tropical climates, while other plants inhabited in the Polar areas, where sunlight is not present. From marketable and dietary point of view Poaceae is very important family of plants for the welfare of human beings, as its many members are known as staple food and fodder for livestock (Jones, 1999).

Plant categorization was started since emergence of man on this plant to make a distinction for their proper use. Plants were grouped and named on the basis of foodstuff or therapeutic value. With the increase in specific knowledge categorization of plants modified into the science of taxonomy. Grasses tribes are mostly prevalent (Hartley, 1964) but the major fraction of genera (76%) are controlled by a single mass of land (Clayton & Renvoize, 1986). The chief subfamilies named over have been evolved near the beginning on tertiary and develop into broad widen prior to the main breakup of the great continents, through the tertiary periods given by the relatively grasses have low endemism comparison with other plants (Hartley, 1964). Endemism is very common in the continents southern tips of and is partial environments where relict vegetation is permitted to tolerate (Clayton & Renvoize, 1986).

For the identification of all levels of taxonomic ranks (families, tribes, genera and species, etc) the morphological characters provide helpful information.Some taxa of flowering plants are difficult to distinguish on the basis of morphological characters (Nazir *et al*., 2013). The study of the interior parts of plants is, mainly on the basis of the microscopic level is termed as Plant anatomy Evert, 2006). At the cellular level anatomy of plants is used frequently for taxonomic investigation of plants (Raven, 2005 and Wolfgang, 1992). In the study area of Bhimber Azad Jammu and Kashmir, use of tradional medicines is very common because people dwell in rural areas and they would like to utilize herbal or botanic drugs. In this study some species of grasses which were medicinally or economically important selected for research. In this work, main aim of the study to: (i) enlist ethnobotanical uses and traditional therapeutic applications of these grass taxa (ii) to use taximetric approach for proper identification and classification of these selected taxa of grasses and (iii) apply leaf epidermal anatomical and its data of stomatal index for taxonomic authentication.

Materials And Methods

Collection and preservation of plants*:* The research was desigened and carried out at District Bhimber to collect some selected specimens of family Poaceae which were medicinally and also important in life rural people. The samples were collected in triplicate and identified by the taxonomist expert of the Department of Botany, Mirpur Universituy of Science and Technology (MUST) Mirpur Azad Jammu and Kashmir (Pakistan). Sample collection procedure was designed as: first grasses were treated by 10% formalin and bunch was kept in air tight polythene bags. With the help of blotting papers the plants were pressed after collection. For 6-10 days blotting papers were changed every day so that it would saturate water and moisture. Then they were poisoned and mounted sheets having size (24x42 cm) and identified them with the help of Flora of the region and with the Flora of Pakistan (Nasir & Ali, 1970-1989; Ali & Nasir, 1990-1992; Ali & Qaiser, 1992-2007) and confirmed by comparison of samples of herbarium of Mirpur University of Science and Technology Department of Botany Bhimber campus (AJK) following the protocol of Ambasta (2012).

Morphometric analysis: To evaluate the morphological characters five samples per species were selected and their phonetic charaters were measured with the help of binocular light microscope of Modle Stemi, 2000 (Zeiss company). In general 24 quantiative and qualitative morphological characters were studied and used for further analysis. Characters were chosen due to previous studies of authors (Ishtiaq *et al.*, 2001), field observation and different Flora (Davis, 1965)**.** Qualitative and quantitative vegetative and floral features were studied in morphological characters. For achievement of the most precise measurements and finer details, the plant parts were calculated by using solid scale under a dissecting microscope. For this purpose 5X, 10X, 20X magnifiers were used. Under dissecting binocular detailed morphological studies were carried out. Diverse morphological vegetative and floral characters were monitored and established by Flora of Pakistan (Nazir, 2013). Morphological features of plant species were calculated and 5-7 sample of each species were observed for morphological studies (Ahmad, 2009; Chaudhari *et al.*, 2013). Following vegetative and floral charaters were studied and used for taximetric analysis of the selected grasses: Among vegetative characters habit of plant annual or perennial, erect, prostrate or decumbent. Culm height, pubescence of culms, texture of nodes and internodes. Shape, length and width of leaves, texture of lamina, colour and texture of leaf sheath, Length and shape of ligule (Ahmad, 2009). While among reproductive characters inflorescence length, shape of panicle, raceme or spike (open, contracted, cylindrical or ovate) was measured. Spikelet shape, length and width was also measured, pedicellate or sessile and single or in group. Glumes shape, length and width was also measured**.** Lemmas shape, length and width was also measured. Paleas shape, length, width was also measured. Stamens number, size and colour, number of style, stigma, size and colour were also observed. Length, width, pubescence and shape caryopsis was also measured (Ahmad, 2009).

Leaf epidermal anatomy: For anatomical studies fresh leaves of plants were used. Samples of leaves were prepared asadapted by Cotton (1974) who followed Clark (1960) technique.

Stomatal index: Stomatal index of all the plants was also calculated the percentage of number of total stomata per total number of epidermal cells. The stomatal number on both sides upper and lower epidermis was counted and epidermal cells were also counted. The data were analysed and tabulated by given formula:

$$\text{Stomatal Index} = \frac{S}{E \times S} \times 100$$

Anatomical analysis: For anatomical analysis healthy fresh leaves were taken in triplicate and cleaned, washed with double distil water. These leaves were cut with help of blade. The sections were first discolored with Analine blue for 3-4 minutes. Analine blue stain was cleaned off and the sections were counter stained with the help of Safranine solution for 2 minutes and then dried out with pure xylene at interval for few seconds. Then the sections were lastly seen under light microscope and then pictures were taken.

Results

The present research work on selected taxa of grasses was conducted during years 2012 -2014 from District Bhimber of Azad Jammu and Kashmir. This work consisted of taxonomical analysis of some grasses species that are *Cynodon dactylon* L. *Saccharum spontanium* L. *Saccharum ravennae* L. *Poa annua* L. and *Poa nemoralis* L. by employing numerical taxonomic and leaf epidermal anatomy approaches.

Ethnombotaical analysis: The sledction of these taxa of grasses was based on their ethnobotanical and traditional therapeutic uses in rural communities of the area. As the dwellers of the study area mostly belong to villages and hilly areas so the plants are part and parcel of their life. The importance of these members of family Poaceae was explored by using questionaire method based on open-ended and closed-ended interviews from indigenous people. The interviewees were both male and female with age rnage of 20-80 years. The data was collected and subjected to further analysis to confirm the ethnobotanical information of the interviewees (Table 1). This analysis that many speices of the grasses have been known to be source of traditional medicines for human being and for livestock too. These informant consensus factor (ICF) was determined to find the maximum use of these species against different diseass and it was found the ethnobotanical data can be verified by use of ICF as statistical tool. ICF was found highest with value of 1.00 for Dyspepsia and least range for kidney stone pain with relibality range of 0.07 (Table 2).

Table 1. Frequency of respondents explored through questioniree method.

Sr. No.	Interviewee information	Frequency (%)
	Static rank:	
1.	Settler	13.00
	Native	87.00
	Gender:	
2.	Female	33.00
	male	67.00
	Literacy rate:	
	Illiterate	22.00
	University	3.00
3.	College	13.00
	High school	12.00
	Middle school	20.00
	Primary school	30.00
	Marital status:	
4.	Married	42.00
	Single	58.00
	Age:	
	10-15 years	8.00
	16-25 years	17.00
5.	26-35 years	20.00
	36-45years	12.00
	46-55years	8.00
	61- above years	35.00

Soil analysis of study area: The soil analysis of three sampling sites was conducted to explore that the is soil composition and also to find out whether was it appropriate for plants' healthy growth. The soil samples of three areas viz: Bhimber, Barnala and Samahni was collected and analyzed in soil testing laboratory of Agriculture Department of District Bhimber. The analysis depicted that texture of soil of Bhimber was Sandy loam, soil of Barnala was loam and soil of Samahni had clay loam feature. Saturation of soils of three was Bhimber, 32%, Barnala 32% and Samahni 26%. Total soluble salts (TSS) in Bhimber soil sample was 0.3%, in Barnala soil sample it was 0.2% and in Samahni soil samples it showed value of 0.1%. Organic matter in soil was as: Bhimber 0.77%, Barnala 0.68% and Samahni 0.53%. Quantity of Potassium (K) in Bhimber soil sample was 124ppm, in Barnala soil it was 115ppm and in Samahni soil it was 104ppm. Quantity of Phosphorus (P) in Bhimber soil sample was 12ppm, in Barnala soil sample was 11ppm and in Samahni soil sample was 7ppm.

Water analysis of the study area: Water analysis of three sampling sites was done to check the plants' potential to grow and compete in the community. The pH of water sample of Bhimber was recorded 6.8. Suspension was not found. Microbs were present that help plants to conversion of complex molecules into simpler one. The transparency of water was also fair. The pH of water sample of Barnala was recorded 6.3. Microbes were also present. Suspension was found. The transparency of water was fair. The pH of water sample of Samahni was recorded 6.2. Microbes were present but not frequent. The transparency of water was also weak in water sample of Samahni.

Economic potential of grasses for indgigenous communityies of the area: Grasses had a great economic importance for a community in the world. To visualize the economic potential of these grasses for the local people of the area was designed and analyzed by measuring the parameters of plants such as: plants height, length and width of leaves, length of inflorescence, dry and wet weight of leaves and number of leaves per plant were calculated to check the potential of plant species (Table 3 and Fig. 1).

Taximetirc analysis of grasses: All the selected five grasses belong three geenra of family Poaceae. Out of these *Cynodon dactylon* L. belongs to Subfamily Chloridoideae, *Saccharum spontanium* L., *Saccharum ravennae* belongs to Subfamily Panicoideae and *Poa annua* and *Poa nemoralis* L. belongs to SubFamily Pooideae. Among these grasses *Cynodon dactylon* L. belongs to Tribe Chlorideae, *Saccharum spontanium* L., *Saccharum ravennae* belongs to Tribe Andropogoneae and *Poa annua* and *Poa nemoralis* L. belongs to Tribe Poeae. All of these are ethnobotanically important, used in daly needs of life and for curing different ailments by the inhabitants of the area.

To study the differences among all plants the sampling was done from three sites from District Bhimber, (Bhimber, Barnala and Samahni). From each sampling site three samples of each plant were taken to study the differences among species and also the differences among plants of species. Cluster analysis was applied and the genetic distance was calculated to differentiate among all of these through statistical technique.

Table 2. Informant consensus factor (ICF) conducted for diseases treated in the research area.

S. No.	Class	Species	All species (%)	Use citation	All citation (%)	ICF
1.	Dyspepsia	10	8.33	11	8.02	0.10
2.	Lecuoderma	11	9.16	13	9.48	0.16
3.	Fever, whooping cough	15	12.5	16	11.67	0.06
4.	Gynecological troubles	13	8.3	17	12.40	0.25
5.	Diabetes	11	9.16	13	9.48	0.16
6.	Sexual weakness	09	7.5	11	8.02	0.2
7.	Kidney stones	14	11.66	15	10.94	0.07
8.	Skin infections	17	14.16	19	13.86	0.11
9.	Piles	11	9.16	12	8.75	0.09
10.	Anticancer	09	5.83	10	7.29	0.11

Table 3. Analysis of economic potential of the selected grasses for local communities of Distirct Bhimber of Azad Jammu and Kashmir.

Sampling site	Plants	Sample of plants	Height of plant (cm)	Length of leaves (cm)	Width of leaves (mm)	Length of inflorescence (cm)	Wet weight of leaves (g)	Dry weight of leaves (g)	No of leaves per plant
		P1	15.5	6.5	2	5.5	0.18	0.10	21
	Cynodon dactylon L.	P2	13.5	6	0.5	5	0.19	0.11	20
		P3	12.5	7.5	2	6	0.18	0.10	19
		P1	170	50	7	55	1.45	0.51	63
	Saccharum spontanium L.	P2	160	45	6.8	52.5	1.40	0.49	62
		P3	165	48	6.5	53	1.35	0.40	61
		P1	200	100	15	75	0.65	0.32	49
Bh	*Saccharum ravennae* L.	P2	190.5	98.5	14.5	72.5	0.64	0.31	47
		P3	195	99	14	74	0.63	0.30	48
		P1	40.5	10	4	30	0.29	0.17	10
	Poa annua L.	P2	45	9.5	3.8	28	0.27	0.15	11
		P3	41	9	3.5	29.5	0.28	0.16	10
		P1	69	8	5	18	0.33	0.18	27
	Poa nemoralis L.	P2	66.5	7.9	4.8	17.5	0.31	0.19	28
		P3	65	7.5	4.5	17	0.32	0.19	26
		P1	11	4.5	0.5	3.2	0.16	0.7	18
	Cynodon dactylon L.	P2	11.5	3.5	o.6	3.5	0.15	0.6	19
		P3	10.5	5.5	1.5	4	0.16	0.7	16
		P1	90	16	4.6	30.9	0.98	0.35	53
	Saccharum spontanium L.	P2	88	24	3.9	30.8	0.95	0.34	52
		P3	99	25	4.9	40.2	0.96	0.35	54
		P1	125	55	5.7	25	0.59	0.26	44
Br	*Saccharum ravennae* L.	P2	121.5	53	6	26.5	0.60	0.30	45
		P3	130	53.5	5.8	24.5	0.59	0.29	44
		P1	25.5	2.5	1.8	11.5	0.26	0.13	8
	Poa annua L.	P2	26	3	1.5	10	0.24	0.12	7
		P3	29	2.8	2	13	0.25	0.13	7
		P1	41.5	5.1	1.7	6.1	0.30	0.15	25
	Poa nemoralis L.	P2	42	5	1	6	0.31	0.16	24
		P3	43	5.2	1.4	6.2	0.30	0.15	25
		P1	16.5	7	o.5	5	0.13	0.7	16
	Cynodon dactylon L.	P2	17	7	1.9	5.5	0.14	0.8	14
		P3	17	4.5	0.8	5.8	0.12	0.6	15
		P1	140	35	5.8	44.5	0.89	0.34	53
	Saccharum spontanium L.	P2	138	34	5.3	43.6	0.88	0.33	52
		P3	135	33	5	44.8	0.86	0.32	51
		P1	150	70.5	9	55	0.49	0.22	40
Sm	*Saccharum ravennae* L.	P2	145	72.5	9.5	56.5	0.55	0.25	39
		P3	144.5	70	8.7	54.5	0.50	0.24	41
		P1	35	5.5	2.7	22.5	0.21	0.13	6
	Poa annua L.	P2	34.5	5.6	2.7	23.5	0.22	0.14	5
		P3	35	5.7	2.8	23	0.16	0.8	6
		P1	50.5	6.2	3.5	9.5	0.26	0.13	21
	Poa nemoralis L.	P2	52	6	3	10	0.27	0.14	20
		P3	51	6.3	3.1	10.2	0.29	0.15	21

(Key:Bh=Bhimber; Br=Barnala; Sm=Samahni)

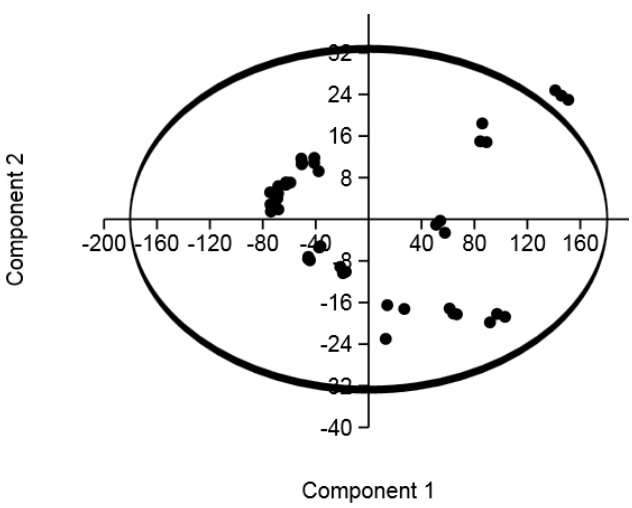

Fig. 1. PCA Depciting Economic potential of grasses for the local communities of District Bhimber Azad Jammu and Kashmir.

Morphogenetics analysis of grasses from Bhimber Zone: The plants collected from a sampling site also exhibited differences among different species and among the different plant populations of the same species. Three samples of each plant were collected and observed the readings werw Tabulated in matrix form (Table 5). The cluster analysis was also done to calculate the genetic distance (GD) between the species of an area and also between the same species. Taxonomically genotypes were divided into two clusters having 135 GD. Cluster-1 and cluster-2 were subdivided into two lineage. Subcluster 1-a and 1-b had genetic distance 37 GD. Subcluster-1b is divided into two lines having 20 GD between *Poa annua* and *Poa nemoralis.* Sub cluster 2 is divided into two lines having 30 GD (Fig. 2).

Morphogenetics analysis of grasses from Samahni Zone: The plants collected from a sampling site also exhibited differences among different species and among the different

plant of a same species. Three samples of each plant were collected observed and readings were doucmented in Table 5. Taxonomically genotypes were divided into two clusters (Cluster I, II) with genetic distance (GD) 108. The cluster-I was divided into two sub clusters. The cluster analysis was also done to calculate the genetic distance (GD) between the species of an area and also between the same species. The genetic distance between two clusters 1 and 2 was 107 GD. Cluster-1 was subdivided into sub clusters1a and1b having 26 GD. Subcluster-1b was divided into sub lineage having 15 GD between two *poa species*. Cluster-2 was subdivided into two lineage having 11 GD between two *Saccharum species*(Fig. 2).

Morphogenetics analysis of grasses from Barnala Zone: The plants collected from a sampling site also exhibited differences among different species and among the different plant of a same species. Three samples of each plant were collected and observed the readings are taken in Table 4. The cluster analysis was also done to calculate the genetic distance between the species of an area and also between the same species.Genetic distance between cluster 1 and 2 was 83 GD. Cluster-1 was divided into two subclusters 1a and 1b having genetic distance 22 GD.Subcluster-1b is sub divided into two lineage having15 GD between two apecies of *Poa* (Fig. 2).

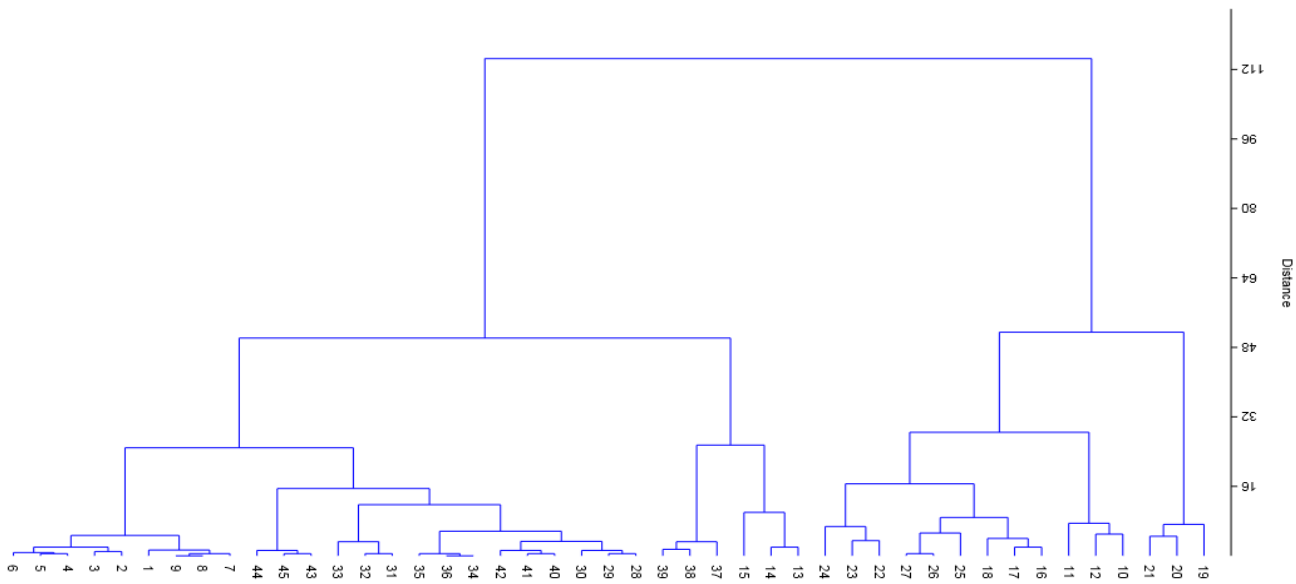

Fig. 2. Morphogenetics Cluster Analysis Based on Species Differences of Selected Plants from District Bhimber AJK
Key: 1= *C. dactylon* plant1 from Bhimber, 2= *C. dactylon* plant2 from Bhimber, 3= *C. dactylon* plant3 from Bhimber, 4= *C. dactylon* plant1 from Barnala, 5= *C. dactylon* plant2 from Barnala, 6= *C. dactylon* plant3 from Barnala, 7= *C. dactylon* plant1 from Samahni, 8= *C. dactylon* plant2 from Samahni, 9=*C. dactylon* plant3 from Samahni, 10= *S. spontanium* plant1 from Bhimber, 11= *S. spontanium* plant2 from Bhimber, 12= *S. spontanium* plant3 from Bhimber, 13= *S. spontanium* plant1 from Barnala, 14= *S. spontanium* plant2 from Barnala, 15= *S. spontanium* plant3 from Barnala, 16= *S. spontanium* plant1 from Samahni, 17= *S. spontanium* plant2 from Samahni, 18= *S. spontanium* plant3 from Samahni, 19= *S. ravennae* plant1 from Bhimber, 20= *S. ravennae* plant2 from Bhimber, 21= *S. ravennae* plant3 from Bhimber, 22= *S. ravennae* plant1 from Barnala, 23= *S. ravennae* plant2 from Barnala, 24= *S. ravennae* plant3 from Barnala, 25= *S. ravennae* plant1 from Samahni, 26= *S. ravennae* plant2 from Samahni, 27= *S. ravennae* plant3 from Samahni, 28= *P.annua* plant1 from Bhimber, 29=*P.annua* plant2 from Bhimber, 30= *P.annua* plant3 from Bhimber, 31= *P.annua* plant1 from Barnala, 32= *P.annua* plant2 from Barnala, 33= *P.annua* plant3 from Barnala, 34= *P.annua* plant1 from Samahni, 35= *P.annua* plant2 from Samahni, 36= *P.annua* plant3 from Samahni, 37= *P. nemoralis* plant1 from Bhimber, 38= *P. nemoralis* plant2 from Bhimber, 39= *P. nemoralis* plant3 from Bhimber, 40= *P. nemoralis* plant1 from Barnala, 41= *P. nemoralis* plant2 from Barnala, 42= *P. nemoralis* plant3 from Barnala, 43= *P. nemoralis* plant1 from Samahni, 44= *P. nemoralis* plant2 from Samahni, 45= *P. nemoralis* plant3 from Samahni.

Taxnomical analysis by using anatomical methods: In anatomical studies leaf epidermal anatomy of grasses was studied. Leaf epidermal anatomy of five grasses was observed from both adaxial and abaxial surfaces.

Leaf epidermal anatomy of *Cynodon dactylon* L.

Adaxial intercostal zone: Long cells 19.03 –29 µm long and 4.5–7 µm wide. Between two costal zones 6-9 rows of long cells present. Between the two costal zones, 2–3 stomatal rows present. Paracytic type of stomata having dumb bell shaped guard cells is present. Subsidiary cells are triangular in shape, 23.75–25 µm long and 12.5–17.5 µm wide. Microhairs and macrohairs absent. Hooks absent.

Adaxial costal zone: Saddle shape silica bodies are present. Silica bodies have 2–4 layers that are 4–6.5 µm long and 3.5–5 µm wide. Short cells having sinuous walls, 13.25–17 µm long and 7–9 µm wide (Fig. 3a).

Abaxial intercostal zone: Long cells having thin sinuous walls, 29–42µm long and 7.5–10.5µm wide. Between two costal zones, 3–5 rows of long cells are present. Between two costal zones, 2–3 stomatal rows are present. Paracytic type of stomata having dumb bell shaped guard cells is present. Subsidiary cells are 13–15 µm long and 10–12 µm wide in dome shape. Microhairs and macrohairs absent. Hooks absent.

Abaxial costal zone: Silica bodies saddle shaped, 6–8 µm horizontally and vertical diameter 9–11 µm. Short cells having sinuous walls, 13–15 µm long and 5–9 µm wide. Rounded bodies (cork cells) present between the short cells and silica bodies, 7–9 µm long and 6 –8 µm wide. Prickles pointed at the tip, 40–42.5 µm long and 10–13 µm wide (Fig. 3b).

Table 4. Morphogenetic Data of Selected Grass Species Used for Taximetirc Analysis from District Bhimber of Azad Jammu and Kashmir.

S. No.	Family	Genus	Plants	S. site	Samples of plants	Plant height (cm)	Length of leaves (cm)	Width of leaves (mm)	Length of ligule (mm)	Length of inflorescence (cm)	Length of spikelet (mm)	Length of glume (mm)	Length of lemmas (mm)
					1	15.5	6.5	2	0.4	5.5	5	2	2
				Bh	2	13.5	6	0.5	o.3	5	4.5	1.5	1.9
					3	12.5	7.5	2	0.4	6	4.8	2	2
					4	11	4.5	0.5	o.1	3.2	3.6	1.2	1.7
1.		*Cynodon*	*C. d*	Br	5	11.5	3.5	o.6	o.1	3.5	3.8	0.8	1.8
					6	10.5	5.5	1.5	0.2	4	3.5	1.1	1.7
					7	16.5	7	o.5	0.3	5	3.9	1.5	2
				Sm	8	17	7	1.9	0.3	5.5	4	1.9	2
					9	17	4.5	0.8	0.2	5.8	3.5	1.7	1.9
					10	170	50	7	1.5	55	7	2.5	2
				Bh	11	160	45	6.8	1.3	52.5	6.9	2.4	1.9
					12	165	48	6.5	1.3	53	6.7	2.2	1.8
					13	90	16	4.6	0.7	30.9	3.8	1.2	0.9
2.			*S.s*	Br	14	88	24	3.9	0.8	30.8	3	1	1.1
					15	99	25	4.9	0.9	40.2	3.5	1.3	1.3
					16	140	35	5.8	1.4	44.5	5.9	1.6	1.8
				Sm	17	138	34	5.3	1.2	43.6	5.3	1.9	1.8
		Saccharum			18	135	33	5	0.9	44.8	5.5	1.3	1.7
					19	200	100	15	20	75	7	3	3
				Bh	20	190.5	98.5	14.5	19.5	72.5	6.5	2.9	2.8
					21	195	99	14	19	74	6.7	2.7	2.7
					22	125	55	5.7	5.7	25	4.5	1.2	1.5
3.	Poaceae		*S. r*	Br	23	121.5	53	6	5.5	26.5	4.7	1.3	1.4
					24	130	53.5	5.8	5.8	24.5	4	1.2	1.2
					25	150	70.5	9	7.5	55	5.2	1.6	2.5
				Sm	26	145	72.5	9.5	7.8	56.5	5	1.9	2
					27	144.5	70	8.7	7	54.5	5.3	1.7	2.1
					28	39.5	10	4	3	30	10	4	3
				Bh	29	39	9.5	3.8	2.9	28	9.6	3.5	2.9
					30	38	9	3.5	2.5	29.5	8.5	3.6	2.8
					31	25.5	2.5	1.8	2.1	11.5	2.6	1.5	2.1
4.			*P. a*	Br	32	26	3	1.5	2.3	10	2.8	1.8	2.2
					33	29	2.8	2	2.1	13	3	1.5	2.1
					34	35	5.5	2.7	2.5	22.5	5.5	3	2.3
				Sm	35	34.5	5.6	2.7	2.6	23.5	5.6	2.9	2.2
		Poa			36	35	5.7	2.8	2.5	23	6	2.8	2.1
					37	69	8	5	2	18	6.5	2	3
				Bh	38	66.5	7.9	4.8	1.9	17.5	6.3	1.9	2.8
					39	65	7.5	4.5	2	17	6.2	1.7	2.9
					40	41.5	5.1	1.7	1.1	6.1	2.5	1.1	2.2
5.			*P.n*	Br	41	42	5	1	1	6	2.4	1	2.1
					42	43	5.2	1.4	1.2	6.2	2.3	1.2	2.2
					43	50.5	6.2	3.5	1.6	9.5	4.5	1.8	2.5
				Sm	44	52	6	3	1.4	10	4.8	1.4	2.4
					45	51	6.3	3.1	1.6	10.2	4.6	1.2	2.5

***** C= *Cynodon dactylon L.*, Ss= *Saccharum spontanium L.,* Sr= *Saccharum ravennae,* Pa= *Poa annua L.*, Pn = *Poa nemoralis L.* P=Plant, Bh= Bhimber, Br=Barnala, Sm=Samahni

Leaf epidermal anatomy *of Saccharum spontanium* L.

Adaxial intercostal zone: Long cells with straight walls, 54–125 µm long and 8–13 µm wide. Between two costal zones 3–8 rows of long cells were present. Between two costal zones was 1–3 stomatal rows were present. Paractic type of stomata present. Subsidiary cells 20-30 µm long and 12-20 µm wide and triangular or low dome shaped. In intercostal zone frequent, bicelled microhairs are present. Macrohairs absent. Hooks absent.

Adaxial costal zone: Silica bodies were cross shaped or intermediate between cross and dumbbell shaped, 7-24 µm long and 3–6.5 µm wide. Angular prickles were present at the margins (Fig. 4a).

Abaxial intercostal zone: Long cells with thin sinuous walls, 61–129 µm long and 7–10µm wide. Between two costal zones, 4–8 rows of long cells were present. Between two costal zones, 2–3 stomatal rows were present. Paractic type of stomata present. Subsidiary cells 25–30 µm long and 10–25 µm wide and triangular in shape. Bicelled Microhairs were seen tapering to pointed apices. Macrohairs absent. Hooks absent.

Abaxial costal zone: Silica bodies are 7-20 µm long and 3–10µm wide, dumbbell shaped. Short cells frequent in the costal zone. Prickles present (Fig. 4b).

Leaf epidermal anatomy *of Saccharum ravennae* L.

Adaxial intercostal zone: Adaxial intercostal long cells having thin sinuous walls, 22.5–37.5 µm long and 7.5–10 µm wide. Number of rows of long cells between two costal zones is 7–9. Number of stomatal rows between two costal zones, 2–3. Paractic type of stomata was present having subsidiary cells of triangular in shape, 19–22 µm long and 11–15 µm wide. Guard cells were dumb bell shape. Microhairs and macrohairs absent. Hooks absent.

Adaxial costal zone: Silica bodies saddle shaped having 3–9 µm long and 5–7 µm wide, 1–3 layers of silica bodies. Short cells with sinuous walls, 13–17 µm long and 6–10 µm wide. Between silica bodies and short cells Rounded bodies were founded 5–7.5 µm long and 6.75–7.25 wide. Prickles present (Fig. 5a).

Abaxial intercostal zone: Long cells with thin sinuous walls, 40–55 µm long and 19–14.5 µm wide. Between two costal zones, 5–8 rows of long cells were present. Between two costal zones, 1–3 stomatal rows were present. Paractic type of stomata was present having subsidiary cells of dome shape, 14–17µm long and 13–15 µm wide. Guard cells were of dumbbell shaped. Microhairs absent. Macrohairs absent. Hooks absent.

Abaxial costal zone: Silica bodies 6–8µm long and vertical diameter 9–12 µm wide. Silica bodies are of saddle shape. Short cells 12–18 µm long and 7–11µm wide. Rounded bodies were seen between the short cells and silica bodies. Prickles were present (Fig. 5b).

Leaf epidermal anatomy of *Poa annua* L.

Adaxial intercostals zone: Long cells were 130-280 µm long and 21–28 µm wide. Between two costal zones, 3–6 rows of long cells were present. Between two costal zones 1–3 stomatal rows were present. Paractic type of stomata having subsidiary cells of dome shaped, 26 – 30µm long and 12–19 µm wide. Microhairs and macrohairs absent. Hooks absent.

Adaxial costal zone: Extended silica bodies were seen. At the margin of costal zones the angular prickles were seen (Fig. 6a).

Abaxial intercostal zone: Long cells were 60–120 µm long and 8–15 µm wide. Between two costal zones 5–12 rows of long cells are present. Between two costal zones 1- 2 stomatal rows were present. Paractic type of stomata was present having subsidiary cells of dome shape, 30–45 µm long and 20–25 µm wide. Microhairs and macrohairs absent. Hooks absent.

Abaxial costal zone: Elongated Silica bodies seen. Long cells present in the costal zone, 35–43 µm long and 3–7 µm wide. Prickles were absent (Fig. 6b).

Leaf Epidermal Anatomy of *Poa nemoralis* L.

Adaxial intercostals zone: Long cells were 101-112 µm long and 15-21 µm wide. Between two costal zones, 6–17 rows of long cells were present. Between two costal zones 1–3 stomatal rows were present. Paractic type of stomata was present having subsidiary cells are parallel, 25–31 µm long and 9.5–15 µm wide. Microhairs and macrohairs absent. Hooks absent

Adaxial costal zone: Elongated silica bodies with straight walls were present. Long cells were also seen in costal zone. Angular prickles present at the margins, 52.5–65 µm long and 12-15.5 wide (Fig. 7a).

Abaxial intercostals zone: Long cells were 111–186 µm long and 13–17 µm wide. Between two costal zones 3–5 rows of long cells were present. Number of stomatal rows between two costal zones, 1–3. Paractic type of stomata having subsidiary cells of parallel sided, dome shape, 13–27 µm long and 17–19 µm wide. Microhairs absent. Macrohairs present. Hooks absent.

Abaxial costal zone: Elongated Silica bodies were 75–190µm long and 8-11 µm wide. Prickles were absent (Fig. 7b).

Stomatal index: During the upper epidermal study of the plants highest stomatal index was found in *S. spontaneous* L. (23.6), after that *P. nemoralis* L. stomatal index is (20.4), *C. dactylon* L. shows stomatal index of (15), *S. ravennae* L. (12.6) and the lowest stomatal index of upper epidermis was found in *P. annua* L. (12.5) (Table 6).

Among the lower epidermal study of stomatal index following results were obtained. *C. dactylon* L. showed the highest Stomatal index (39.7), *S. spontanium* had 32.7, *P. nemoralis* L. (32), *P. annua* L. (18.09) and the lowest stomatal index was found in lower epidermis *S. ravennae* (16.16) Table 2.

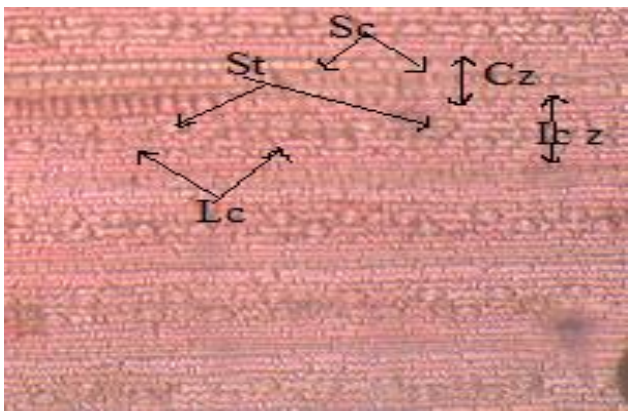

Fig. 3a. Leaf epidermal adaxial surface of *Cynodon dactyolon*.

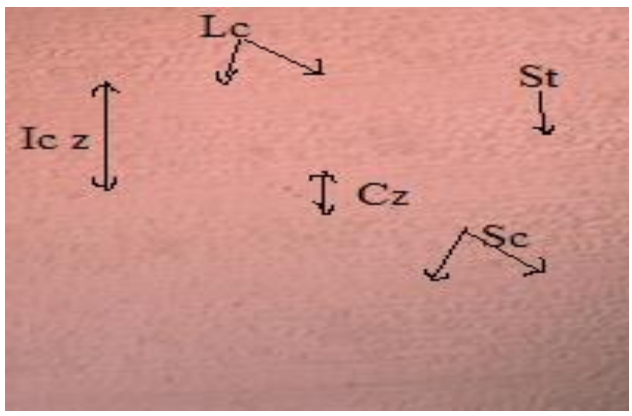

Fig. 3b. Leaf epidermal abaxial surface of C*ynodon dactyolon*.

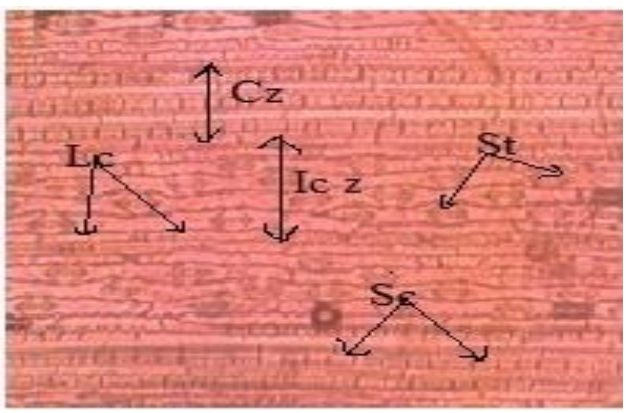

Fig. 4a. Leaf epidermal adaxial surface of *Saccharum spontanium*.

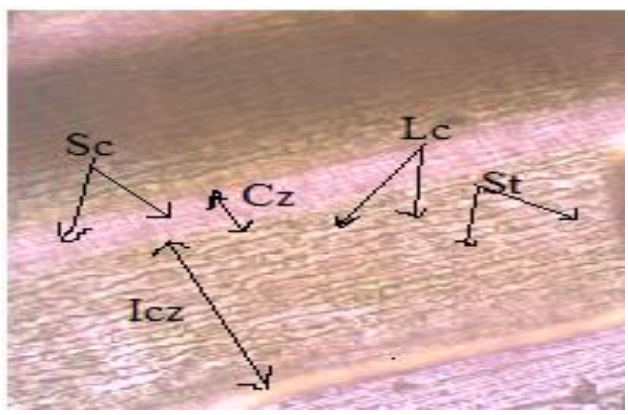

Fig. 4b. Leaf epidermal abaxial surface of *Saccharum spontanium*.

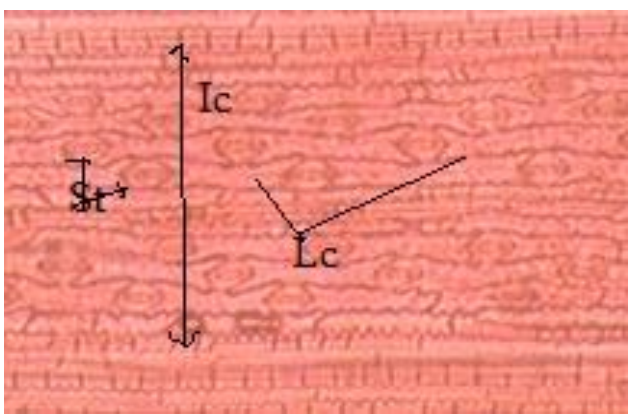

Fig. 5a. Leaf epidermal adaxial surface of *Saccharum ravennvae*.

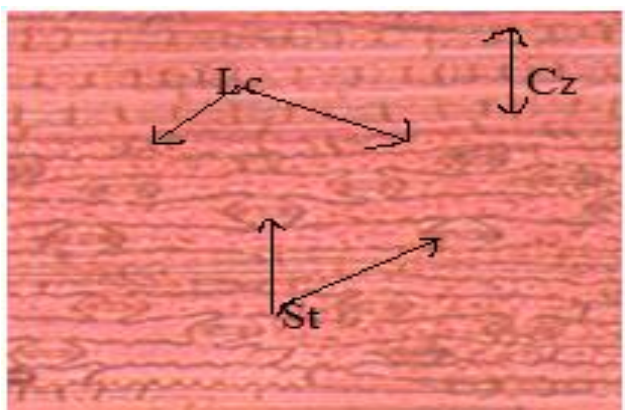

Fig. 5b. Leaf epidermal abaxial surface of *Saccharum ravennae*.

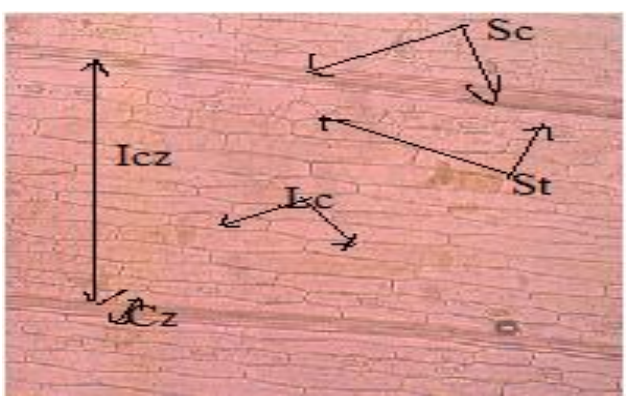

Fig. 6a. Leaf epidermal adaxial surface of *Poa annua* L.

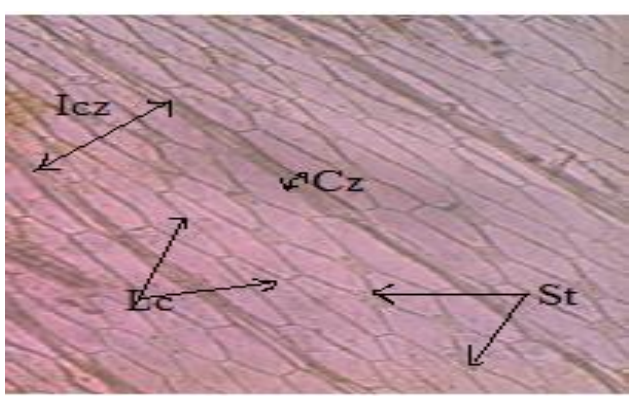

Fig. 6b. Leaf epidermal abaxial surface of *Poa annua* L.

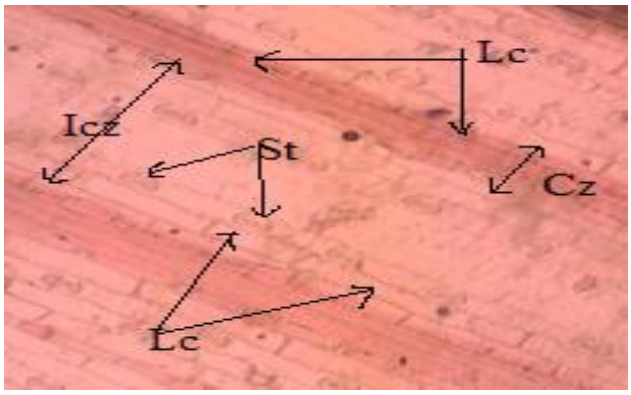

Fig. 7a. Adaxial leaf epidermal surface of *Poa nemoralis* L.

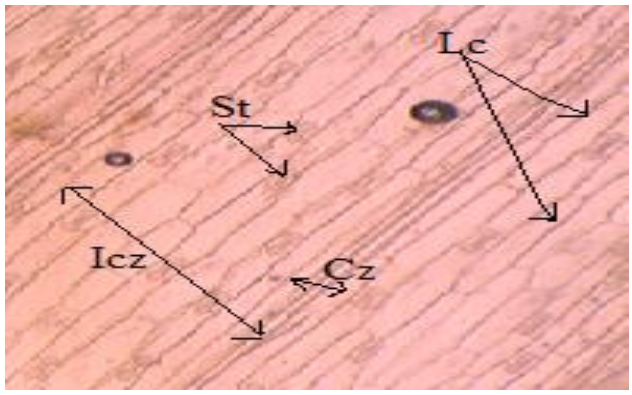

Fig. 7b. Abaxial leaf epidermal surface of *Poa nemoralis* L.

Key: St= stomata, Sc =short cells, Cz =costal zone, Icz=inter costal zone, Lc=long cells,

Table 5. Leaf epidermal anatomical studies of selected grasses from district Bhimber AzaD Jammu and Kashmir.

S. No.	Plant name	Long cells		Short cells		Stomata		Prickle hairs		Hooks		Silica bodies		Micro hairs		Macro hairs	
		AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD	AB	AD
1.	*Cynodon dactylon* L.	6-9 r	6-9 r	P	P	2-3 r	2-3 r	A	A	A	A	P	P	A	A	A	A
2.	*Saccharum spontanium* L.	4-8 r	4-10 r	Fr	P	2-3 r	1-3 r	A	P	A	A	P	P	A	P	A	A
3.	*Saccharum ravennae* L.	6-10 r	8-11 r	p	P	1-3 r	2-3 r	P	P	A	A	P	P	A	A	A	A
4.	*Poa annua* L.	3-5 r	4-8 r	P	P	2-4 r	2-4 r	A	P	A	A	P	P	A	A	A	A
5.	*Poa nemoralis* L.	8-17 r	7-15 r	P	P	1-3 r	1-3 r	A	P	A	A	P	P	A	A	A	A

* AB= Abaxial, AD= Adaxial, A= absent, p = present, r =rows, fr = frequent

Table 6. Stomatal index of upper and lower epidermis of selected plants from District Bhimber AJK.

S. No.	Plants	Type of stomata		No. of stomatal cells (S)		No of epidermal cells (E)		Stomatal index (I)	
		UE	LE	UE	LE	UE	LE	UE	LE
1.	*Cynodon dactylon* L.	Paractic	Paractic	16	33	90	150	15	39.7
2.	*Saccharum spontanium* L.	Paractic	Paractic	27	53	87	109	23.6	32.7
3.	*Saccharum ravennae* L.	Paractic	Paractic	13	27	90	140	12.6	16.16
4.	*Poa annua* L.	Paractic	Paractic	8	19	56	86	12.5	18.09
5.	*Poa nemoralis* L.	Paractic	Paractic	10	24	35	51	20.4	32

The plants of study area were subjected to cluster analysis using MVSP software which divided samples of medicinal grasses into two main clusters named as cluster-I (which had plants of Tehsil Bhimber and Tehsil Barnanal) while cluster-II had plants grouped together from Samahni area. These plants have been separated on basis of their morphometric features. The plants of Samahni had different morphology than plants of Bhimber and Barnala. Then each of the cluster was subdivided into sub-clusters based on their micro-differential characters. These all features are depicted in the Fig. 8 of analysis. The genetic distance of species of bhimber was also calculated. Cluster-1 having 135 GD was divided into two subclusters. Genetic distances between 1a and 1b was 37 GD. While subcluster-2a and 2b had genetic distance 30 GD. Sub cluster 1b was divided into two sub lineage having genetic distance 20 GD. This GD shows that the species studied close relationship among themselves.

Identification key based on morphological characters

1 + Sheath membraneous/ciliate, ligule present with flat leaves ……...……...…… 2
- Sheath membrane galcous present with glabrous leaves ……...…….. 3
2 + Inflorescence digitate 3-6cm in length ovate, curved and purplish spikelets ……...… *Cynodon dactylon* L.
- Inflorescence panicle 30-55cm in length, pedicelled, silky white …..……...… *Saccharum spontanium* L.
3 + Leaves glbrous, truncate ligule, inflorescence panicle, heteromorphous inflorescence …..……...…… 4
- Leaves glbrous, blunt ligule, inflorescence panicle ……...……...…….. 5
4 + Heteromorphous inflorescence, yellowish spikelets, 20-75cm in length ....……...… *Saccharum ravennae* L.
- Inflroesence Panicle-Open, -12cmin length, Oblong, lanceolate spikelets ..……..…… *Poa annua* L.
5 Truncate ligule,leaves boat shape, panicle open 6-18cm in length, ovate, oblong spikelets ...…… *Poa nemoralis* L.

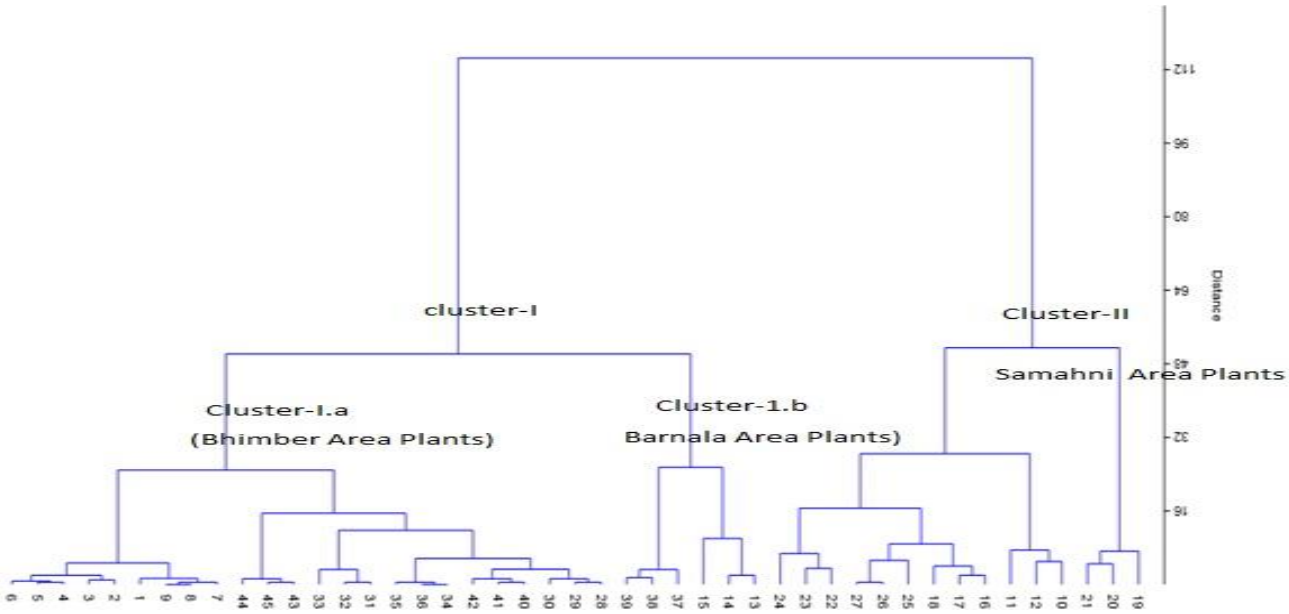

Fig. 8. Morphogenetics (Taximetrics) Cluster Analysis of Five Grassess from District Bhimber of AJK (Describing distributions of taxa on Inter-genera, Inter-species and inter-ecoregions basis).

Disscusion

In present research work taxonomy of wild grasses which have medicinal importance in local herbal cure system have been done from District Bhimber using morphometric approach and sophisticated analytical tools. The qualitative and quantitative morphological and leaf epidermal study was conducted. Present study revealed that all the species observed contained paracytic type of stomata in which guard cells were accompanied by two subsidiary cells and guard cells were dumb bell shaped. The anatomical features of of five grasses have been evaluated during the present study. Abaxial leaves epidermal anatomical studies were taken place which revealed that numbers of rows of long cells were greater in *Poa annua L.* that were 8-17 where as *Saccharum ravennae* had 6-10 rows, *Cynodon dactylon* L. had 6-9 rows, *Saccharum spontaniu* L. had 4-8 rows and *Poa nemoralis* L. had 3-5 rows of lon cells. Number of rows of stomata were also counted that were present stomatal rows were present in greater quantity in *Poa annua* L. as 2-4 rows while in *Cynodon dactylon* L. and *Saccharum spontanium* L. stomatal rows were 2-3 rows, and in *Saccharum ravennae* and *Poa nemoralis* L. 1-3 stomatal rows were present. Micro hairs were not shown in all grasses. Prickles hairs were present only in *Saccharum ravennae*. Hooks and macro hairs were not seen. In table 4.3 adaxial leaf epidermal anatomical studies were done which showed variation on adaxial leaves surface Chaudhari *et al.,* (2014).

Micro hairs and macro hairs were absent in *Poa annua* L. while silica bodies were in elongated shape, dome shape subsidiary cells, prickle hairs. Guard cells found to be low dome shaped or parallel sided similar to the previous results obtained by (Metclafe, 1960; Peterson *et al*., 2006; Ahmad, 2009). According to Metcalfe (1960) and Ellis (1986) Silica bodies did not have any defined shape in P*oa sp.* But present study revealed that in *Poa annua* L .silica bodies were in elongated shape that was similar to the study of Chaudhari *et al.,* (2014). They reported that shape of silica bodies was elongated. Nazir, (2013) also conducted similar research fodder species of Poaceae from Potohar region of Pakistan. In *Poa annua* L. microhairs were absent. Costal silica bodies were horizontally elongated sinuous. Stomata were common and 45-54 µm long that show similarity to the present study.

The present studies prove that medicinal grasses can be identified morphologically using gross phonetic features. This plants of samples from Samahni have separate lineage assembled in cluster –II that is due to their better growth due to presence of more humidity and better organic materials in soil. While plants of Barnala and Bhimber areas are although of two separate tehsils due to similar environmental features. However, these environmental features do have an impact on genetics and expressed genome in form morphology strcutrues (Ahmad *et al.,* 2009).

Conclusion

These plant species have great economic and medicinal potential. These plants species required to be identified and classified which is conducted first time for these taxa from this region. These studies will be helpful for agriculturists and layman (farmers) which describe that which species have food availability in which area (fields) so that community can develop better economic potential from available resource. After antimicrobial study it is depicted that these grasses are also important in medicinal point of view. So communities have to available resources. Results revealed the presence of large variations in epidermal cells configuration at different taxonomic levels. On the type and shape of leaf epidermal cells further comprehensive work is needed for their amplification as a taxonomic character. The plants extracts showed diverse degrees of antimicrobial activity

on the microorganisms. The bioactive substances obtained from these plants can be used in the formulation of antimicrobial agents for the treatment of various bacterial diseases. Results revealed the presence of large variations in epidermal cells configuration at different taxonomic levels. The differences were observed in the abaxial and adaxial surfaces of the leaf of *Cynodon dactylon* L., *Saccharum spontanium* L., *Saccharum ravennae, Poa annua,* and *Poa nemoralis* L. It is concluded that yet there is a need of more comprehensive work on the shape and type of leaf epidermal cells for their further elaboration as a taxonomic character.

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