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PHENOTYPIC INTRASPECIFIC DIVERSITY EXPLORATION AMONG THE ACCESSIONS OF *CYMBOPOGON CITRATUS* (D.C.) STAPF FROM ASIA AND AMERICAN SUB-CONTINENT THROUGH MULTIVARIATE TECHNIQUES

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ABSTRACT

Cymbopogon citratus (D.C.) Stapf belongs to family Poaceae, plant of warm climatic conditions. However genetic diversity of this species is yet to explore. Phenotypic variation is representation of genotypic variation. Therefore, tropical and subtropical regions of Pakistan were explored for collection of germplasm. In the present study morphological data of 50 accessions were collected; 49 accessions were from Pakistan whereas 1 from USDA. These 50 accessions were cultivated for consecutively two years to find out the most acclimatized accession for cultivation in Pakistan. The 8 qualitative (Stem type; Leaf length; Leaf blade; Leaf sheath; Leaf shape; Leaf arrangement; Leaf tips; Leaf margins). and 13 quantitative markers (Number of tillers; Plant height; Leaf length at early stage, Leaf width at early stage, Leaf length at mature stage, Leaf width at mature stage, Leaf sheath length at early stage, Leaf sheath width at early stage, Leaf sheath length at mature stage, Leaf sheath width at mature stage, Number of leaves per tiller and Fresh and dry weight of leaves.) were observed for consecutive two years 2017-2018 and 2018-2019 to select the most diverse accession. The data was analyzed through statistical analysis software principal component analysis and XL STAT. All accessions did not behave alike in both years because of environmental fluctuation. However, the accessions 38452 (Alipurchatha) 384516(Faisalabad) and 38457(Khairpur) were most diverse and significant with reference to yield.

Key words: Germplasm, Morphological markers, Cultivation, Environment, Correlation, Cluster, Diversity.

INTRODUCTION

Cymbopogon citratus (D.C.) Stapf is commonly known as lemongrass. It is a tropical aromatic grass that grows up to a height of 6 feet, having bulbous stem and glabrous leaf (Shah *et al*., 2011). . This genus is represented by 140 species. They are found in tropical and semi-tropical regions of Asia, America and Africa. About 45 species are widely distributed in subcontinent, out of which eight species are found in Pakistan (Kak and Kaul, 1997). *C. citratus* can grow in hot and humid environment but it requires adequate sunshine for its optimum growth. The life span of *C. citratus* is nearly 6 months sowing and harvesting time is from March to September whereas natural revival for this crop is upto 8 years through tiller.Vaqar *et al.* (2007). Economical and medicinal worth of this species is because of essential oil. *C. citratus* oil includes 16 chemicals among which geranial, neral and myrcene are main constituents (Gbenou *et al.,* 2013).

C. citratus, leaves are the site for essential oil production. The essential oil has a large content of citral, which is used as a raw material for beta-carotene and vitamin A production. Essential oil is extensively used in perfumery, cosmetics and pharmaceutical industries (Costa *et al.,* 2016). Brazilians used lemongrass as medicine for the treatment of gastrointestinal and nervous disorders (Carlini *et al.,* 1986). It is also used as remedy of athlete's foot (Adeneye and Agbaje, 2007).People in Asia, South America and West Africa used its leaves for making tea. Lemongrass tea could be very good substitute of black tea. The use of black tea in Pakistan is very high; hence its import rate has increased in the last few decades. By the year of 2010 Pakistan has become the largest importer of black tea (Latif *et al.,* 2008). However, use of Lemongrass as substitute of black tea could reduce the expenses. Now Lajawab lemongrass tea is being manufactured in Karachi. Moreover green tea is rich in polyphenols and antioxidants (Chen *et al.,* 2002). Pharmaceutically, it is used to prevent oxidative stress related diseases such as cardiovascular diseases, cancer and degenerated diseases (Ogura *et al.,* 2008). Except all of its benefits, there is no cultivation of lemongrass in Pakistan. Only in 1986-88 root- stock of lemon grass were planted in Gilgit (Khan and Ahmad, 1992). For upgradation and conservation of plant, awareness about germplasm diversity is of prime importance (Tripathi and Singh, 2014). Therefore, the present study was undertaken to explore the phenotypic diversity among the accessions of *C. citratus* belonging to different geographical regions of Pakistan. Different morphological markers were used for the analysis of genetic diversity.

MATERIALS AND METHODS

Phenotypic diversity was observed for 50 accessions. Out of those forty-nine accessions were collected from Pakistan and one accession was from the USDA Gene bank. These voucher specimens were submitted to National Agriculture Research Centre (NARC) Islamabad for the allotment of accession numbers.

Cultivation of Germplasm:

The germplasm was planted at Botanic Garden of Lahore College for Women University Lahore. All accessions were properly tagged. High temperature is required for best growth of this plant so germplasm was planted in March and harvested in September progeny (P1). The same experiment was repeated for consecutive two years (2017-18; 2018-19).

Selection of Morphological Markers:

Phenotypical markers were selected by following Flora of Pakistan for Poaceae (Cope, 1985). Ten plants per accession were selected to collect data in consecutive two years to avoid the ambiguity. Eight qualitative (Stem type; Leaf length; Leaf blade; Leaf sheath; Leaf shape; Leaf arrangement; Leaf tips; Leaf margins). and 13 quantitative markers traits (Number of tillers; Plant height; Long leaf length, Long leaf width, Small leaf length, Small leaf width, Long leaf sheath length, Long leaf sheath width, Small leaf sheath length, Small leaf sheath width, Number of leaves per tiller, Fresh and dry weight of leaves.) were selected for present study. The character like Date of Collection (important to identify seasonal variation); Place of Collection (to highlight geographical variations); Accession number; Planting date; Harvesting date; Habit and Habitat were considered to identify the seasonal variation.

Statistically analysis was done by using Cluster analysis and principal component analysis by applying software SPSS and XLSTAT.

RESULTS

In the present study 50 accessions of *Cymbopogon citratus* (D.C.) Stapf were investigated (Table 5)

Morphological traits: To elucidate the phenotypic diversity among the *cymbopogoncitratus* germplasm, the morphological traits were studied. Over all qualitative traits did not show remarkable diversity but the quantitative traits were significant diverse.

Number of Tillers: Only one tiller was planted of all accessions of *C. citratus* in 2018 and after 6 months the number of tillers was counted. The germplasm showed remarkable diversity. The maximum number of tiller was observed in accession of 38457 (Khairpur) 24 followed by 38452 (Alipurchatha) 21, 384516 (Faisalabad) 17, 384519 (Islamabad) 21and 384543 (Dera Ismail Khan) 22. The minimum number of tiller was observed in accessions 38451 (Swat) 3, followed by 38456 (USA) 3, 38458 (Sawabi) 2, 384512 (Lahore) 4 and 384549 (Rajanpur) 4 in first year data. In the next year the maximum number of tillers was observed in accession of 38457 (Khairpur) 46 followed by 38452 (Alipurchatha) 43, 384531 (Karachi) 40, 384516 (Faisalabad) 38, 38453 (Gujranwala) and 384539 (Depalpur). The minimum number of tillers was observed in accessions of 384525 (Kohat) 3 and 384523 (Hafizaabad) is 4 (Table 4).

Number of Leaves: The number of leaves were counted and variation was observed. The maximum number of leaves was in accession 38452 (Alipurchatha) 21 followed by 384511 (Lahore) 18 and 384516 (Faisalabad) 16. The minimum number of leaves was observed in accession 384525 (Kohat) and 384538 (Kamoki) 5. In the next year (2019) the maximum number of leaves was observed in accession 38452 (Alipurchatha) 23 and minimum in accessions of 384525 (Kohat) 4 and 384549 (Rajanpur) is 5 (Table 4).

Leaf length at early stage: The leaf length and width of each accession were measured by measuring tape at early and mature stage of plant growth. In 2018 the maximum leaf length was observed in accession 384517 (Faisalabad) 81.28cm followed by 38452 (Alipurchatha) 76.25cm, 38459 (Phalia) 71.12 cm and 384525 (Peshawar) 63.56cm accessions also showed good leaf length. The minimum growth was found in the accessions of 384523 (Pindi) 12.72 cm and 38451 (Swat) 17.78cm. In 2019 the maximum leaf length was observed in accession of 38452 (Alipurchatha) 78.27cm and 384517 (Faisalabad) 76.98cm. The minimum leaf length was observed in accessions of 384541 (Daska) 21.23cm followed by 384511 (Lahore) 22.34cm and 384549 (Rajanpur) 22.86cm (Table 4).

Table 1. Principal component analysis for quantitative agro-morphological traits in *C.citratus* for 2018 data.

	F1	F2	F3	F4	F5
Eigenvalue	3.981	1.944	1.347	1.302	1.201
Variability (%)	30.626	14.957	10.360	10.014	9.239
Cumulative %	30.626	45.584	55.944	65.958	75.198
Eigenvectors:					
	F1	F2	F3	F4	F5
Tillers	0.121	-0.063	-0.616	0.297	-0.100
Leaf No.	0.261	-0.388	0.065	-0.182	-0.004
LL Length	0.329	0.464	-0.053	-0.168	0.075
LL Width	0.279	0.083	0.507	0.027	-0.158
SL Length	0.282	0.162	-0.138	0.491	-0.135
SL Width	0.160	0.036	0.282	0.684	-0.013
LLS Length	0.327	0.238	-0.063	-0.276	-0.112
LLS Width	0.284	-0.198	0.305	0.096	0.290
SLS Length	0.173	-0.225	0.225	-0.155	-0.582
SLS Width	0.039	0.050	0.143	-0.017	0.686
Plant Height	0.368	0.436	-0.098	-0.158	0.013
Fresh Wt.	0.370	-0.367	-0.181	-0.050	0.117
Dry Wt.	0.366	-0.351	-0.217	-0.059	0.146

Table 2. Correlation among quantitative agro-morphological traits of *C.citratus* for 2018 data.

Variables	Tillers	Leaf No.	LL Length	LL Width	SL Length	SL Width	LLS Length	LLS Width	SLS Length	SLS Width	Plant Height	Fresh Wt	Dry Wt
Tillers	1												
Leaf No.	0.100	1											
LL Length	0.059	0.057	1										
LL Width	-0.097	0.209	0.366	1									
SL Length	0.357	0.169	0.409	0.226	1								
SL Width	0.000	-0.036	0.053	0.295	0.460	1							
LLS Length	0.058	0.199	0.560	0.277	0.222	0.095	1						
LLS Width	0.034	0.441	0.203	0.396	0.116	0.311	0.199	1					
SLS Length	-0.022	0.378	-0.006	0.348	0.194	-0.006	0.173	0.076	1				
SLS Width	-0.102	0.068	0.105	0.041	0.082	-0.029	-0.050	0.124	-0.147	1			
Plant Height	0.139	0.091	0.933	0.389	0.451	0.091	0.686	0.209	0.060	0.071	1		
Fresh Wt	0.233	0.486	0.190	0.225	0.238	0.152	0.328	0.428	0.265	0.035	0.264	1	
Dry Wt	0.229	0.484	0.205	0.178	0.237	0.139	0.327	0.404	0.209	0.035	0.288	0.983	1

Values in bold are different from 0 with a significance level alpha=0.05

Table 3. Principal component analysis for quantitative agro-morphological traits in *C.citratus* for 2019 data.

Eigenvalues:					
	F1	F2	F3	F4	F5
Eigenvalue	5.265	1.756	1.246	0.960	0.881
Variability (%)	40.496	13.510	9.588	7.386	6.780
Cumulative %	40.496	54.006	63.594	70.980	77.760
Eigenvectors:					
	F1	F2	F3	F4	F5
Tillers	0.319	-0.089	-0.099	0.321	-0.044
Leaf No.	0.305	-0.202	-0.034	-0.116	0.315
LL Length	0.297	0.150	0.092	0.001	-0.496
LL Width	0.226	0.095	-0.453	-0.422	0.283
SL Length	0.236	0.366	-0.147	-0.276	-0.222
SL Width	0.080	0.623	-0.020	-0.287	0.117
LLS Length	0.304	0.090	-0.159	0.255	-0.201
LLS Width	0.298	-0.038	0.433	-0.218	0.138
SLS Length	0.210	0.178	-0.233	0.502	0.561
SLS Width	0.126	0.285	0.691	0.102	0.266
Plant Height	0.354	0.129	-0.019	0.321	-0.255
Fresh Wt	0.345	-0.365	0.039	-0.185	0.016
Dry Wt	0.344	-0.354	0.079	-0.187	-0.006

Table 4. Correlation among quantitative agro-morphological traits of *C.citratus* for 2018 data.

Variables	Tillers	Leaf No.	LL Length	LL Width	SL Length	SL Width	LLS Length	LLS Width	SLS Length	SLS Width	Plant Height	Fresh Wt	Dry Wt
Tillers	**1**												
Leaf No.	**0.388**	**1**											
LL Length	**0.490**	**0.326**	**1**										
LL Width	**0.324**	**0.395**	0.246	**1**									
SL Length	**0.294**	**0.294**	**0.454**	**0.333**	**1**								
SL Width	-0.014	-0.089	0.219	**0.284**	**0.439**	**1**							
LLS Length	**0.444**	**0.418**	**0.427**	**0.364**	**0.332**	0.138	**1**						
LLS Width	**0.355**	**0.479**	**0.430**	0.254	**0.283**	0.094	**0.387**	**1**					
SLS Length	**0.442**	**0.341**	0.187	0.267	0.241	0.212	**0.324**	0.178	**1**				
SLS Width	0.139	0.139	0.220	-0.083	0.127	**0.303**	0.104	**0.464**	0.144	**1**			
Plant Height	**0.585**	**0.433**	**0.617**	**0.291**	**0.460**	0.173	**0.740**	**0.428**	**0.426**	0.269	**1**		
Fresh Wt	**0.567**	**0.617**	**0.413**	**0.346**	0.236	-0.116	**0.404**	**0.552**	0.218	0.065	**0.497**	**1**	
Dry Wt	**0.581**	**0.586**	**0.425**	**0.321**	0.231	-0.093	**0.379**	**0.575**	0.197	0.092	**0.510**	**0.979**	**1**

Values in bold are different from 0 with a significance level alpha=0.05

Leaf width at early stage: In 2018 the maximum leaf width was observed in the accession of 384524 (Peshawar) 1.78cm and 384522 (Sargodha) 1.77cm. The minimum leaf width was observed in the accession of 384550 (Bannu) 0.52cm followed by 38456 (USA) 0.56cm and 384526 (Multan) 0.58cm also showed minimum leaf width. In 2019 the maximum leaf width was observed in the accession of 384524 (Peshawar) 1.77cm and 384517 (Faisalabad). The

minimum leaf width was observed in the accessions of 38453(Gujranwala) 0.25cm, 384549 (Rajanpur) 0.55cm and 384530 (Karachi) 0.56cm (Table 4).

Leaf length at mature stage: In 2018 the maximum leaf length was observed in the accession of 384521 (Bahawalpur) 124.46 cm followed by accession of 384531 (Karachi) 121.92cm, 384526 (Multan), 384518 (Faisalabad), 384522 (Sargodha) 119.38cm, 38452 (Alipurchatha) and 384514 (Patoki) (116.84 cm) also showed the increased in length of leaf. The minimum leaf length was observed in the accession of 384550 (Bannu) 50.86 cm. In 2019 the maximum leaf length was observed in the accession of 384517 (Faisalabad) 134.56 cm. 384521 (Bahawalpur) 126.76 cm, 384514 (Patoki) 121.09 cm and 38452 (Alipurchatha) 120.84 cm. The minimum leaf width was observed in the accession of 384525 (Kohat) 58.46 followed by 38455 (Shiekupura) 59.54 cm and 384542 (Daska) 58.98cm (Table 4).

Leaf width at mature stage: The maximum leaf width was observed in the accession of 384517 (Faisalabad) followed by 38454 (Gujranwalla) 2.31cm and 384514 (Patoki) 2.28cm. The minimum leaf width was observed in the accession 384537 (Layya) 0.76 and384543 (Dera Ismail Khan) 0.78cm after one year of growths**.** In 2019the maximum leaf width was again observed in the accession of 384517 (Faisalabad) 2.56 followed by 38455 (Shiekupura) 2.28 cm, 384519 (Islamabad) 2.08 cm and 384525 (Peshawar) 2.06cm. The minimum leaf width was observed in the accessions of 38453 (Gujranwalla) 0.51cm and 384523 (Pindi) 0.68cm (Table 4).

Leaf sheath length at early stage: In 2018 the maximum sheath length was noted in the accession of 384513 (Patoki) 12.48cm followed by accession of 384511 (Lahore) 11.21cm, 384510 (Kunja) 10.78cm and 38457 (Khairpur) 10.16 cm also showed good growth of sheath length. The minimum sheath length was observed in the accessions of 384534 (Kashmir) 0.57cm and 384537 (Layyah) 0.58cm. In 2019 the accessions of 384513 (Patoki) again showed maximum sheath length (12.62cm) followed by the accessions of 38457 (Khirpur) 10.32cm and 38454 (Gujranwala) 10.16 cm. The minimum sheath length was observed in the accessions of 384536 (Bahawalnager) 0.71cm followed by the accessions of38456 (USA) 1.27cm and 384525 (Kohat) 2.54cm (Table 4).

Leaf sheath width at early stage: In 2018 the maximum sheath width was observed in the accessions of 384511 (Lahore) and 384516 (Faisalabad) 1.77cm followed by the accessions of 384531 (Karachi) 1.02cm and 384546 (Sialkot) 1.01cm. The minimum sheath width was noted in the accession of 384541 (Daska) 0.24cm and 384529 (Karachi) 0.25cm. In 2019 the maximum sheath width was observed in the accession of 384516 (Faisalabad) 1.39cm followed by the accessions of 384520 (Islamabad) (1.29cm) and 38454 (Gujranwala) 1.27cm. The minimum sheath width was noted in the accessions of 384527 (Hafizabad) 0.14cm, 384529 (Karachi) 0.15cm and 384524 (Peshawar) (Table 4).

Leaf Sheath length at mature stage: In 2018 maximum sheath length was observed in the accession of 384541 (Daska) 48.26cm followed by the accession of 38454 (Gujranwala) 43.21 cm and 384540 (Jhelum) 43.18 cm. The minimum sheath length was found in the accessions of 34457 (Khairpur) 17.78cm, 384514 (Patoki) 17.89cm and 38451 (Swat) 19.05cm. In 2019 the maximum sheath length was observed in the accession of 38451 (Swat) 55.88cm followed by the accessions of 384541 Daska (48.27cm) and 384540 (Jhelum) 47.27cm. The minimum sheath length was observed in the accession of 38456 (USA) 12.71 followed by the accessions of 384514 (Patoki) (17.78cm) and 384542 Daska (17.86cm) (Table 4).

Leaf sheath width at mature stage: In 2018 the maximum sheath width was observed in the accessions of 384512 (Lahore) 3.17cm followed by the accessions of 384516 (Faisalabad) 2.54cm and 384520 (Islamabad) 2.13cm. The minimum sheath width was noted in the accessions of 384527 (Hafizabad) 0.51cm, 38457 (Khairpur) 0.58cm and 384550 (Bannu) 0.59cm. In 2019 the maximum sheath width was observed in the accession of 384516 (Faisalabad) 2.54cm followed by the accessions of384520 (Islamabad) 2.12cm and 38452 (Alipurchatha) 1.94cm. The minimum sheath width was noted in the accessions of 384525 (Kohat) 0.51cm and 38456 (USA) 0.58cm (Table 4).

Plant Height: In 2018 the maximum plant height was observed in the accession of 384531 (Karachi) 167.64cm followed by the accessions of 384526 (Multan) 165.16cm, 384522 (Sargodha) 162.56cm and 38452 (Alipurchatha) 160.02cm. The minimum plant height was observed in the accession of (Swat) 66.04cm followed by the accessions of 384550 (Bannu) 71.12cm and 384546(Sialkot) 70.10 cm. In 2019 the maximum plant height was observed in the accessions of 384531 (Karachi) 170.07cm followed by the accessions of 38452 (Alipurchatha) 162.07cm and 38459

(Phalia) 162.56cm. The minimum plant height was noted in the accession of 384525 (Kohat) 68.58cm, 38456 (USA) 73.66cm, and 38458 (Sawabi) 74.66cm (Table 4).

Table No 5. Geographical location and physical factors of accessions

Accession No	Place of Collection	Source	Altitude	Latitude	Temperature	Rainfall
38451	Sawt	Cultivated	975 m	35.2227°N-72.4258°E	16.6°C	866 mm
38452	Alipur Chatha	Wild	419 m	32.2654°N-73.8125°E	23.9°C	578 mm
38453	Gujranwala (Kot shera)	Wild	226 m	32.1877 °N-72.1945°E	23.9°C	578 mm
38454	Gujranwala	Wild	226 m	32.1877 °N-72.1945°E	23.9°C	578 mm
38455	Sheikhupura	Cultivated	236 m	31.7167°N-73.9850°E	24.1°C	476 mm
38456	USA (Virgin Island)	Cultivated	474 m	18.3358° N, 64.8963°W	25.2°C	1200 mm
38457	Khairpur	Wild	61 m	26.8822°N-69.0970°E	26.9°C	99 mm
38458	Swabi	Wild	340 m	34.1241°N-72.4613°E	22.2°C	639 mm
38459	Phalia	Cultivated	205 m	32.4327°N-73.5771°E	24.0 °C	530 mm
384510	Kunja	Wild	23 3m	22.2587°N-71.1924°E	23.8 °C	746 mm
384511	Model Town (Lahore)	Cultivated	21 7m	31.5204°N-74.3587°E	24.1 °C	607 mm
384512	Jail road (Lahore)	Cultivated	217 m	31.5204°N-74.3587°E	24.1 °C	607 mm
384513	Pattoki	Wild	186 m	31.0249°N-73.8479°E	24.3 °C	340 mm
384514	Pattoki	Cultivated	186 m	31.0249°N-73.8479°E	24.3 °C	340 mm
384515	Halla	Wild	178 m	31.1199°N-73.7272°E	24.3 °C	340 mm
384516	Manawala (Faisalabad)	Wild	183 m	31.4504°N-73.1350°E	24.2 °C	346 mm
384517	Chak JB Dhanola(Faisalabad)	Wild	183 m	31.4504°N-73.1350°E	24.2 °C	346 mm
384518	Faisalabad	Cultivated	183 m	31.4504°N-73.1350°E	24.2 °C	346 mm
384519	Bani Gala (Islamabad)	Wild	540 m	33.6844°N-73.0479°E	21.3 °C	941 mm
384520	Islamabad	Cultivated	540 m	33.6844°N-73.0479°E	21.3 °C	941 mm
384521	Bahawalpur	Wild	214 m	29.3544°N-71.6911°E	23.8 °C	187mm
384522	Sargodha	Cultivated	190 m	32.0740°N-72.6861°E	23.8 °C	410 mm
384523	Pindi	Wild	508 m	33.5651°N-73.0169°E	21.5 °C	941 mm
384524	Peshawar	Wild	331 m	34.0151°N-71.5249°E	22.7 °C	384 mm
384525	Kohat	Wild	489 m	33.5889°N-71.4429°E	22.8 °C	529 mm
384526	Multan	Cultivated	122 m	30.1575°N-71.5249°E	25.6 °C	175 mm
384527	Hafizaabad	Wild	200 m	32.0712°N-73.6895°E	24.1 °C	437 mm
384528	Hafizaabad(Jalalpur bhattian)	Wild	200 m	32.0712°N-73.6895°E	24.1 °C	437 mm
384529	Karachi (Karachi University)	Cultivated	8 m	24.8607°N-67.0011°E	25.9 °C	194 mm
384530	Karachi (Mahkoma mosmiat)	Wild	8 m	24.8607°N-67.0011°E	25.9 °C	194 mm
384531	Karachi	Wild	8 m	24.8607°N-67.0011°E	25.9 °C	194 mm
384532	Phull (Karachi)	Cultivated	8 m	24.8607°N-67.0011°E	25.9 °C	194 mm
384533	Shakarghar	Wild	268 m	32.2572°N-75.1604°E	26.2 °C	722 mm
384534	Kashmir	Wild	209 m	33.9259° N, 73.7810°E	21.9 °C	976 mm
384535	Kashmir	Cultivated	209 m	33.9259° N, 73.7810°E	21.9 °C	976 mm
384536	Bahawalnagar	Wild	16 3m	30.0025°N-73.2412°E	25.1 °C	204 mm
384537	Layyah	Wild	143 m	30.9693°N-70.9428°E	25.2 °C	195 mm
384538	Kamoke	Cultivated	201m	31.9765°N-74.2220°E	23.9 °C	573 mm
384539	Depalpur	Wild	167 m	30.6770°N-73.6477°E	24.6 °C	287 mm
384540	Jhelum	Wild	234 m	32.9425°N-73.7257°E	23.6 °C	842 mm
384541	Daska	Wild	217 m	32.3363°N-74.3675°E	23.8 °C	652 mm
384542	Daska	Cultivated	217 m	32.3363°N-74.3675°E	23.8 °C	652 mm
384543	Dera Ismail khan	Wild	165 m	31.8626°N-70.9019°E	24.5 °C	249 mm
384544	Dera Ismail khan	Cultivated	165 m	31.8626°N-70.9019°E	24.5 °C	249 mm
384545	Pindi	Wild	508 m	33.5651°N-73.0169°E	21.5 °C	941 mm
384546	Sialkot	Wild	240 m	32.4945° N, 74.5229°E	23.8 °C	652 mm
384547	Sialkot	Cultivated	240 m	32.4945° N, 74.5229°E	23.8 °C	652 mm
384548	Kasur	Wild	218 m	31.1179° N, 74.4408° E	23.9 °C	424 mm
384549	Rajanpur	Wild	97 m	29.1044° N, 70.3301° E	26.0 °C	205 mm
384550	Bannu	Wild	3806 m	32.9910° N, 70.6455°E	35 °C	249 mm

Fresh weight of Plants: In 2018 the maximum fresh weight of plant was observed in the accessions of 384516(Faisalabad) 3500 g and 38462 (Alipurchatha) 3080g. The minimum fresh weight was noted in the

accessions of 384548 (Kasur) 170g followed by the accessions of384544 (Dera Ismail Khan) 175g and 38456 (USA) 179g. In 2019the maximum fresh weight was noted in the accessions of 38462 (Alipurchatha) 5020g followed by the accessions of 384516 (Faisalabad) 5000g, 384519 (Islamabad) 2080g, and 384526 (Multan) 2000g. The minimum fresh weight was measured in the accession of 384549 (Rajanpur) 180g followed by the accessions of 38458 (Swabi) 210g and 38456 (USA) 287g (Table 4).

Dry weight of plant: In 2018 the maximum dry weight was observed in the accessions of 384516 (Faisalabad) 2300 g and38462 (Alipurchatha) 2060g. The minimum dry weight was observed in the accessions of 384548 (Kasur) 60g followed by the accessions of 384544 (Dera Ismail Khan) 70g and 384542 (Daska) 80g.In 2019 the maximum dry weight was observed in the accessions of 384516 (Faisalabad) 3790 and 38462 (Alipurchatha) 3076 g. The minimum dry weight was noted in the accessions of 384525 (Kohat) 80g followed by the accessions of 38458 (Sawabi) 87g and 38456 (USA) 124g (Table 4).

Principal Component analysis: To reveal the patterns of genetic variability, principal component analysis was carried out on the basis of quantitative agro-morphological traits for 50 accessions all the variables recorded during 2018 (Table 1) and 2019 shown in (Table 3). The maximum variability was found in PCA1 (30.626) and minimum was in PCA 5 (9.239).

Principal Component analysis for 2018 data: In the PCA 1 the maximum variability was observed (30.626). The maximum variation was observes by fresh weight (0.370) and minimum variation was in small leaf sheath width (0.039). There is no negative value in PCA 1. In the PCA 2 the variability was observed (14.957). The maximum variation was shown by long leaf length (0.464). The minimum variation was observed in small leaf width (0.036). The maximum negative value was shown by tiller (-0.063) and minimum negative value was observed in leaf number (-0.388). In the PCA 3 the variability was observed (10.360). The maximum variation was shown by long leaf width (0.507). The minimum variation was observed in leaf number (0.065). The maximum negative value was shown by long leaf length (-0.053) and minimum negative value was observed in tiller (-0.616). In the PCA 4 the variability was observed (10.014). The maximum variation was shown by small leaf width (0.684). The minimum variation was observed in longleaf width (0.027). The maximum negative value was shown by small leaf sheath width (-0.017) and minimum negative value was observed in long leaf sheath length (-0.276). In the PCA 5 the variability was observed (9.238). The maximum variation was shown by small leaf sheath width (0.686). The minimum variation was observed in plant height (0.013). The maximum negative value was shown by leaf number (-0.004) and minimum negative value was observed in small leaf length (-0.135). The graphical illustration (Fig. 1,3) showed the distribution of all accessions on x-axis and y-axis on the basis of similar characters. F-1 contributed (30.63%) whereas F-2 contribution was (14.96%). Overall contribution noted was (45.58%). PCA showed there is no remarkable difference between the local accessions like Sawabi, Swat, Bannu, Depalpur, Shiekupura and international accession of United State Kingdom so these all accessions were placed on the same region. The accessions of Alipurchatha and Faisalabad were placed in the same region because of similarity in morphological marker with each other while showed different character from the rest of accessions. The distribution pattern showed that the placement of different accessions in the same region was not because of single character but due to the contribution of correlation of various agronomical characters.

PCA analysis for 2019 data: In second-year data the maximum variability observed in PCA 1 was (40.496) shown in Table 2. The maximum variation was shown by plant height (0.354) and minimum variation was in small leaf width (0.080). There is again no negative value in PCA 1.In second year data the variability observed in PCA 2 was (13.510). The maximum variation was shown by small leaf width (0.623). The minimum variation was observed in long leaf sheath width (0.090). The maximum negative value was shown by tiller again (-0.089) and minimum negative value was observed in fresh weight (-0.365). In second year, data the variability observed in PCA 3 was (9.588). The maximum variation was shown by small leaf sheath width (0.691). The minimum variation was observed in fresh weight (0.039). The maximum negative value was shown by small leaf width (-0.020) and minimum negative value was observed in long leaf width (-0.453). In the second-year data the variability observed in PCA 4 was (7.386). The maximum variation was shown by small leaf sheath length (0.502). The minimum variation was observed in long leaf length (0.001). The maximum negative value was shown by leaf number (-0.116) and minimum negative value was observed in long leaf width (-0.422). In second year data the variability observed was (6.780). The maximum variation was shown by dry weight (-0.006). The minimum variation was observed in plant height (0.013). The maximum negative value was shown by leaf number (-0.004) and minimum negative value was observed in long leaf length (-0.496). The graphical illustration (Fig. 2,4) showed that the PCA

analysis of 2019 was different from the last year. F- 1 contributed 40.50%, whereas F-2 was at 13.51%. Overall contribution of both factors was observed 54.01%. This time PCA placed many other accessions like Hafizabad, Multan, Gujranwalla etc. in the same region of the accessions Alipurchathaand Faisalabad because of morphological similarity. Likewise, the local accessions of Rajanpur, Kohat, Kunja, Dera Ismail Khan and the international accession of United State Kingdom was placed in the same component because of similarity of morphological characters.

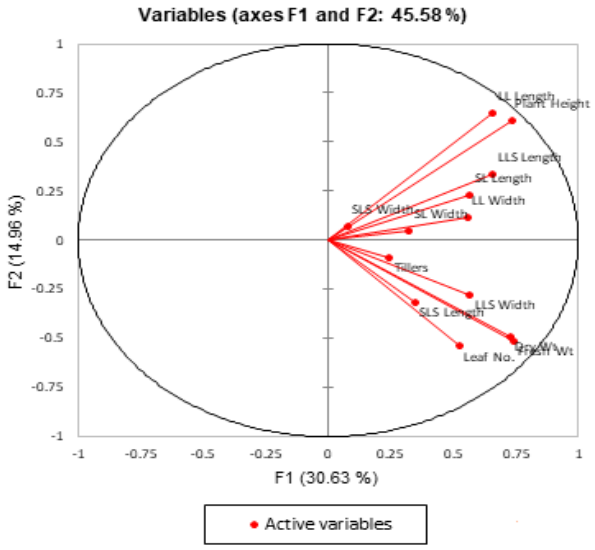

Fig.1. Contribution of quantitative agro-morphological traits in 1st and 2nd principal components in *C.citratus* for 2018 data.

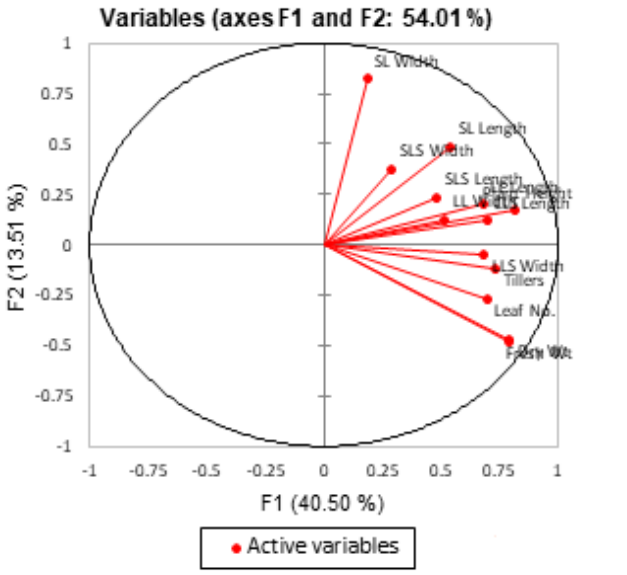

Fig. 2. Contribution of quantitative agro-morphological traits in 1st and 2nd principal components in *C.citratus* for 2019 data.

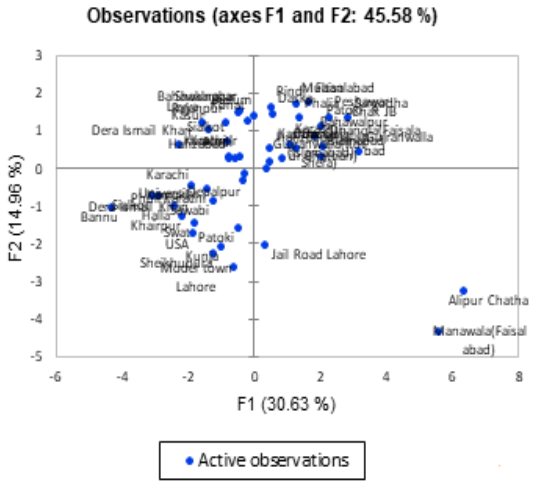

Fig. 3. Graphic illustration of *C.citratus* genotypes based on quantitative agro- morphological traits in PC1 and PC2 for 2018 data.

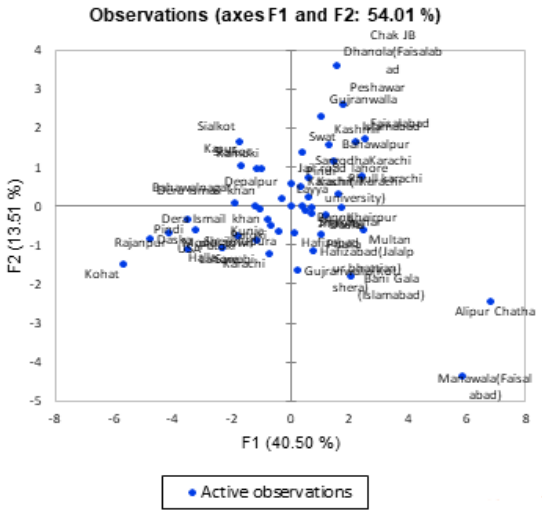

Fig. 4. Graphic illustration of *C.citratus* genotypes based on quantitative agro- morphological traits in PC1 and PC2 for 2019 data.

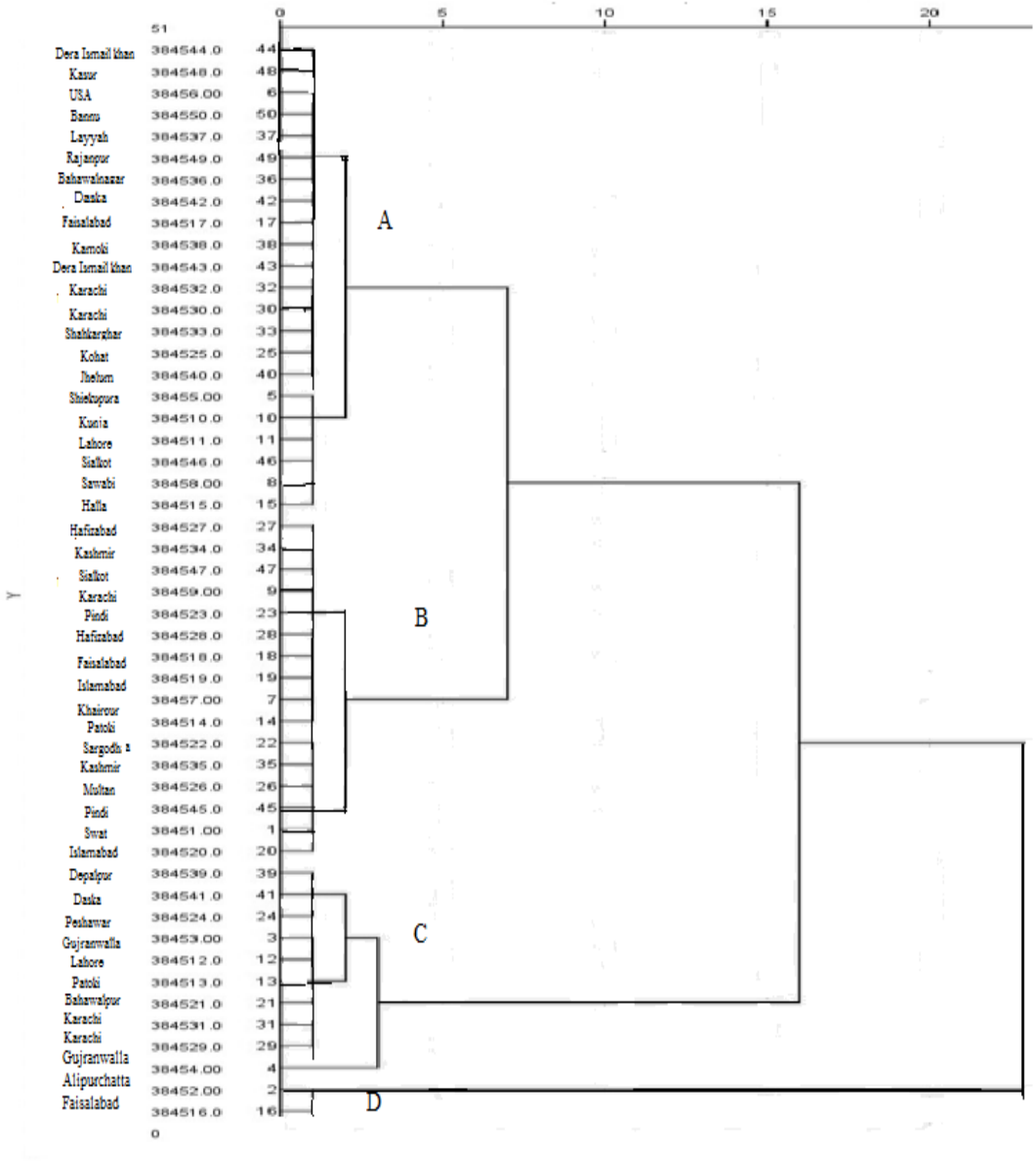

Fig. 5. Dendrogram reflecting the relationship between *C.citratus* genotypes for 2018 data

Correlation: The quantitative agro-morphological traits of 50 accessions and of *C.citratus* were analyzed for elucidating the simple correlation coefficient between them during 2018 and 2019, given in the (Table 2) and (Table 4), respectively. In first year data the maximum positive correlation was found in dry weight and fresh weight (0.98). The minimum positive correlation was found in long leaf sheath width and tiller number (0.034). The maximum negative correlation was found in small leaf sheath length and long leaf length (-0.006). The minimum negative correlation was found in small leaf sheath width and small leaf sheath length (- 0.147).In the second year data the maximum positive correlation was found in dry weight and fresh weight (0.979). The minimum positive correlation was found in fresh weight and small leaf sheath width (0.065). The maximum negative correlation was found in small leaf width and tiller number (-0.014). The minimum negative correlation was found in dry weight and small leaf width (- 0.093).

Cluster Analysis for 2018 data: A total of 50 accessions of *Cymbopogon citratus* were distributed into 4 clusters A, B, C and D through cluster analysis based on agro- morphological traits collected in 2018 shown in (Fig.5).Cluster- A comprises of 22 accessions that belongs to different areas i: e two accessions were belongs to Deraismail Khan, two accessions from Karachi and others belongs to Kasur, USA, Hyderabad, Layyah, Rajanpur, Bahawalnagar, Daska, Faisalabad, Kunja, Kamoki, Shakarghar, Kohat, Jhelum, Shiekupura, Lahore, Sialkot, Sawabi and Halla. The common character in all these accessions to produce a smaller number of tillers i: e (3-8). Cluster –B comprises of 16 accessions that belongs to different areas, two accessions were belongs to Hafizabad, two from Kashmir, two from Islamabad and other belongs to Khairpur, Patoki, Sargodha, Faisalabad, Pindi, Phalia, Sialkot, Multan, Pindi and Swat. The common character in all these accessions to showed maximum value of long leaf length. Cluster- C comprises 10 accessions that belongs to different areas like two accessions were belongs to Gujranwalla, two collected from Karachi, and other belongs to, Lahore, Patoki, Bahawalpur, Peshawar, Depalpur and Daska. The all 10 accessions showed maximum fresh weight. Cluster – D comprises of two accessions that belongs to Alipurchatha and Fasialabad. These two accessions showed maximum dry weight.

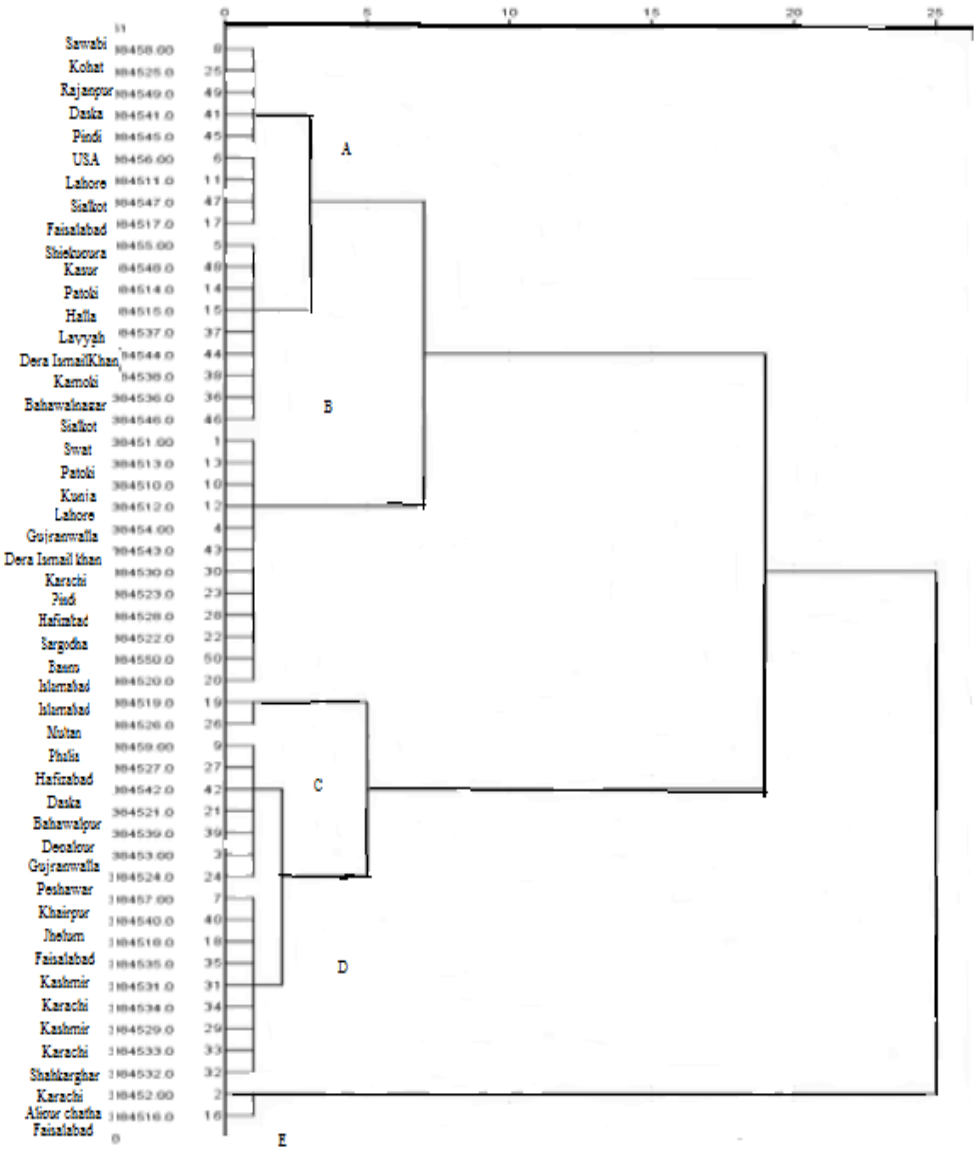

Fig. 6. Dendrogram reflecting the relationship between *C.citratus* genotypes for 2019 data.

Cluster Analysis for 2019 data: A total of 50 accessions of *Cymbopogon citratus* were again distributed into 5 clusters A, B, C ,D and E through cluster analysis based on agro- morphological traits collected in 2019 shown in (Fig.6).Cluster- A comprises of 18 accessions that belongs to different areas i: e two accessions from Sialkot and others belongs to Kasur, USA, Pindi, Layyah, Rajanpur, Bahawalnagar, Daska, Faisalabad, Kamoki, Kohat, Shiekupura, Sawabi, Dera Ismail Khan, Patoki, Lahore and Halla. These 18 accessions produce a smaller number of leaves in 2019.Cluster –B comprises of 12 accessions that belongs to different areas. These accessions were belongs to Hafizabad, Swat, Patoki, Gujranwalla, Kunja, Lahore, Sargodha, Islamabad, Karachi, Dera Ismail Khan, Bannu, Pindi. These accessions showed approximately same long leaf sheath width. Cluster- C consists of 2 accessions that belong to Faisalabad and Multan. These two accessions were showed maximum dry weight. Cluster- D comprises of 16 accessions. These accessions were belongs to different areas i:e three accessions were belongs to Karachi, two from Kashmir and other belongs to Gujranwalla, Khairpur, Faisalabad, Bahawalpur, Peshawar, Hafizabad, Shakarghar, Depalpur, Jhelum, Phalia and Daska. Theses accessions were produced maximum number of tillers. Cluster –E comprises of two accessions that belongs to Alipurchatha and Fasialabad. These two accessions showed maximum Fresh and dry weight in 2019.

DISCUSSION

Plant morphology is physical form of plant. To form a profile of plants morphological study can be applied on any specie of plant by studying its vegetative and reproductive parts. It helps to identify the varieties by comparison within the species also make the general comparison of plant species that showed similar structure. The comparative study of plant morphology is the backbone of plant systematic and helps to explore the diversity, evolution and phylogeny of plant. Endress *et al.* (2000). The species of *Cymbopogon* showed great variation in morphological and anatomical traits Nath *et al.* (2002). The characterization of agronomical character is a main step towards phenotypic variability and genetic diversity Koffi *et al.* (2008). In the present study germplasm of *C.citratus* that was collected from different areas of Pakistan were evaluated for consecutive two years to explore the phenotypic diversity.

Different agronomical characters were studied to explore the variation in member of family Poaceae like leaf color, leaf shape and plant height etc. In the present study qualitative character (leaf color, leaf blade, leaf shape, leaf venation, leaf arrangement, leaf type and stem type) and quantitative characters (number of tillers, number of leaves per tiller, long leaf length, small leaf length, long leaf width, small leaf width, long leaf sheath length, long leaf sheath width, small leaf sheath length, small leaf sheath width, plant height, fresh weight and dry weight) were study to explore the morphological variation within the specie of *C.citratus.*

Qualitative traits showed minute or no environmental effects. Therefore, no remarkable modifications were found in qualitative traits recoded in two years. The quantitative traits are economically important and showed reasonably variation. Iqbal *et al.* (2015) evaluated 153 genotypes of *Zea mays* that were collected from different countries to elucidate the phenotypic diversity on the basis of agro-morphological traits. He recorded the height of maize ranged from 71.9 to 226.1 cm. Like this in the present study 50 accessions of *C.citratus* were collected from different areas of Pakistan to explore the genetic diversity on the basis of agro-morphological traits. Plant height was measured during consecutive two years 2018 and 2 019 so great level of variation in plant height was found between different accessions of *C. citratus.* It ranged from 67cm to 168cm. This variation in plant height may be due to different genotype or origin of collection because plant development and its growth depend upon the endogenous and environmental signals. Different growth regulators that called plant hormones involve in the growth of plants like auxin trigger the plant height. Gray (2004). So the accessions that showed the maximum plant height have more concentration of auxin than the accessions showed small height.

Tiller number is important character of grasses. Lafitte *et al.* (2001) worked on rice which also belongs to poacea. He used 135 accessions of rice to evaluate the root traits on the basis of vegetative characters he noted the variation in tiller number per plant and maximum number that found was 21. Similarly, 1 tiller of each accession of *C.citratus* was planted at sowing time and great variation was found in tiller number at harvesting time. It ranged from 2 to 56. The accession of Khairpur produce 21 tillers in first year of growth while in the second year tiller number exceed to 56 which is highest number among all accessions. It showed that environment affects the morphological traits also the plant hormones play an important role to regulate the number of tillers. According to Cai *et al.* (2018) zeatin is responsible to increase the number of tillers in wheat so it might be the possible reason of the increase in number of tillers in some accessions of *C.citratus.*

Gyenis *et al.* (2007) did comparison of wild barley and modern barley cultivars on the basis of agronomical traits he noted the leaf width of barley that range from 0.3 to 0.9cm. Similarly in the present study leaf width of all accessions of *C.citratus* was noted and it ranged from 0.5 to 2.54 cm. According to Barišić *et al.* (2006) intensity of

light affects the plant growth. If the light intensity is low it enhanced the plant height, leaf length and width but if the light intensity is higher it suppressed the plant height and leaf size. So it could be the possible reason of variation in leaf width.

Principal component analysis and cluster analysis was applied on the present study to understand the diversity among the germplasm of *C.citratus*. PCA analysis distributed all germplasm into 5 components (PCA-1 to PCA-5) in both years. There is no negative value was found in PCA-1 of both years. Ramakrishnan *et al.* (2016) collected 60 accessions of Guinea grass and applied Principle component analysis to know the contribution of morphological traits in variability. He found that these characters can be used to set the criteria to improve the fodder yield. Similarly in the present study Principle component analysis was applied on the 50 accessions of *C.citratus* and found the diversity in morphological traits. It helps to understand which accessions can be used for cultivation to increase the yield.

Cluster analysis was applied on all the germplasm of *C.citratus* to find out the similarity and correlation. Like accessions that were belong to different origin Gujranwalla, Karachi, Lahore, Patoki, Bahawalpur, Peshawar, Depalpur and Daska were present in the same cluster. These accessions showed maximum fresh weight because these accessions have more potential to adopt the different environment. In the second year some accessions behave differently and different results were obtain among cluster like accessions collected from Faisalabad and Multan showed maximum dry weigh that could be due to environmental variations. Correlation plays an important role for the selection of traits for breeding purpose. During both years different correlation was found but the consistency in positive correlation was found in fresh and dry weight. Likewise Iqbal *et al.* (2015) found the positive correlation among the grain yield and plant height.

It was observed that morphological variations noted and composed for all two years were not constant among all accessions. This is because of some environmental changes. In general the accessions belonging to Faisalabad, Alipurchatha and Khairpur were showed good growth in both years. *C.citratus* is not cultivated on large scale so the data accumulated from all these accessions could be helpful for formers to choose the best accession for cultivation.

Conclusion: The accessions grown for two years showed morphological diversity. It was observed that accessions belonging to Faisalabad, Alipurchatha and Khairpur were more diverse. So it was concluded that these accessions can be used for cultivation.

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