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Selection of Elite Lines from *Durum* through Morphomatric and Proteomic Markers

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Abstract: *Exploration of landraces is beneficial to the modern agriculture, which broaden the gene-pool for the plant breeders and enhance crop performance. In this work we inspect the genetic variability of 104 local Triticum durum landraces collected from Malakand division through Agro-morphological and biochemical markers. High phenotypic variability observed in both qualitative and quantitates traits. Experimental field were designed for Agro-Morphological parameters planted at Botanical garden University of Malakand District Dir lower. There were no diseases found in all landraces. Correlation analysis revealed that days to germination was positive strongly significant correlated to the days to leaf emergence (DLE) and (DSE) days to spike emergence with (DLE) days to leaf emergence (0.637** & 0.699**) while 100 seed weight was significantly positively correlated with Days to leaf emergence (0.469**) and Seed storage proteins were detected through Sodium Dodecyl Sulphate, Polyacrylamide Gel Electrophoresis (SDS-PAGE). The high level of variation was noted in B1 (0.90 %) followed by B13 (0.80 %), B5 (0.65 %), B12 (0.65 %), B2 (0.60 %) and B7 (0.60 %) polymorphism respectively. Similarly, B14 (0.20%) revealed low level of i.e. B10 (0.20%) B11 (0.25%), B9 (0.30%), B3 (0.40%), B8 (0.45%), B4 (0.50%), and B6 (0.55%). Similarly, results revealed that low level of variation was found in B14 (0.20 %), B10 (0.20 %), B11 (0.25 %), B9 (0.30 %), B3 (0.40 %), B8 (0.45 %) and B4 (0.40 %).*

Keywords: *Triticum durum, Genetic diversity, Agro-Morphology, SDS-PAGE*

INTRODUCTION

At the present time durum wheat is of major significance in cereal areas of the Mediterranean basin, being mostly cultivated under rainfed environment (Nachit, 1998). Durum wheat is mainly used in pasta manufacturing, but it is also the raw material for the manufacture of other traditional products of Mediterranean countries as flat breads, couscous and bulgur, and in Italy and the Middle East, even for bread-making (Kovacs et al. 1993, 1995).From the early decades of the 20th century, and similarly to what happened in bread wheat and barley, the coming on of new and homogeneous varieties lead to the progressive abandon of the locally-adapted, unimproved and genetically more diverse landraces. Ancestry analyses approved out on varieties derived as of this germplasm indicated to, in several cases, the genetic backdrop beneath these flourishing varieties was tapered (Maccaferri et al. in press). Numerous research approaches have been urbanized for assessing genetic diversity. Agronomical and morphological character have been expansively used (Jain et al.

1975; Porceddu 1976). The loss of the diversity vulnerable by plant breeders (genetic erosion) and the requirement of introgression more deviation have become matters of concern during the last decade (Peccetti et al. 2001). Exceptional material, such as landraces, which are no longer cultivated, although still existing in the gene banks, is useful to broaden the genetic basis of propagation programs (Allard, 1996). Mediterranean durum landraces have been establish to comprise enviable traits absent in enhanced cultivars (Nachit, 1992). The proficient deployment of germplasm assortment is improved by the familiarity of the patterns of genetic variation within the assortment for particular traits of attention (Sorrells et al., 1993). They are quite simple to quantify, and frequently helpful to further purposes as well.

Materials and Methods

Research Area

The Research was conducted at Botanical Garden and Herbarium University of Malakand in Dir. L District, Khyber Pakhtunkhwa Pakistan, from November to February. Temperatures range between 8.4C° and 26.1C°.

Morphological characterization

The taken Morphological data consist on both Quantitative and Qualitative characters. The Qualitative characters including spike color , plant orientation, stem color and seed color, while the Quantitative characters are germination days, flowering days, leaf emergence days, spike emergence days, height of total plant, spike's number per plant, size of spike, weight of 100 grain, yield of grains, total plant biomass (gm) and harvest index (IPGRI, 1994).

Biochemical Characterizations

To estimate the level of Genetic diversity at protein level the following method were followed by using SDS-PAGE. Seed of every landrace was grinded in to fine powder on mortar and pestle and 0.01 gm of this seed flour taken in 1.5ml in Eppendorf tubes. Protein extraction buffer (300 micro liters) was added to 0.01 gram seed flour of each genotype with the help of micropipette. Bromophenole blue (BPB) as well added to the protein extraction buffer as attracting pigment to observe proteins in the gel. After addition of protein extraction buffer to 0.01 gm grind seed added to Eppendorf tubes, vertexes carefully to mix the grind seed with the proteins extraction Buffer. After vertex the sample were incubated at 70 C° for four hours. The Homogenized sample was centrifuged at 14,000 rpm upto 15 minutes on 40 C° and stored at 20C° in refrigerator. After centrifugation the homogenized sample were again incubated at 70C° for fifteen minutes. Two plates of gel was arranged at the same time for Electrophoresis, carefully cleaned the internal sides of glass plates used for electrophoresis with 70% Ethanol. Placed seal gastekson glass plates with space. Fix a set of glass plates by a clip. Prepared the separation and stacking gel as follows, the use of separation gel was resolution of poly peptide bands on molecular weight basis. 2 tiny gels was set for this reason. After preparation, pour the separation gel into the gap between the glass plates. Small amount of distilled water is than added to separation gel softly to avoid gel face from air as well as promote complex. Then gel were placed for about 30 minutes until proper solidification of gel and then prepared stacking gel. The stacking gel uses for to keep the loaded sample as well as separate well for subsequently movement of separation.

After preparation of stacking gel the distilled water take out from the peak of separation gel. Then pour stacking gel on separation gel and put combs on stacking gel. Wait about twenty minutes; remove chips, seal gasket as well as combs. The electrophoresis procedure was carried out by the method of Laemmle (1970) Using 11.5% poly acrylamide gel with miner modification that was using 15% polyacrylamide gel. Electrode buffer (0.025 M Tris, 0.129 M Glycine, 0.125% SDS) were added to

the base pool of the tools. The gel plates were placed in the revelation taking think about that heremust not be air fizz at the base of the gel. The electrode buffer solution were poured on the peak pool of the tools, wash down gel with syringe. A total amount of 20 micro liter of protein extract solution be loaded in all well by the help of micro pipette. Tools were associated through constant electric supply (100v) until the bromophenol Blue (BPB) was reached to the base of gel plate. After the process of electrophoresis the plates was positioned on the slab to take apart the glass plates from the gel. The distilled water be added with separation gel for simple eliminations of plates, the gel was stained in staining solution upto 10 minutes above a shaker, after that wash with distilled water in a tray. The gels was then shifted to another tray having de-staining solution and kept until the back ground of the gel become clear. After de-staining the gel was kept dipped in distilled water for one hour and a small amount of spirit were added to fix banding pattern. Next the gels were fixing via using plastic sheet for unending storage.And the molecular data was recorded on the basis of presence and absences of proteins bands i.e. "1" for the presence and "0" for the absence of bands. The intensity of bands was considered as major and minor bands I.e., The high intensity glowing band as major and low intensity glowing bands as minor. Different software such as excel, SPSS and for cluster Analysis was carried out using software PC-ORD.

Results

Qualitative traits

A significant level of variation was observed in qualitative traits, On the base of spike density the lines of *durum* show variation from each other, on the base of spike density there are three types of spike density (lax, intermediate, and dense) were observed, during the present study the lax and intermediate were observe but dense were not appear, the frequency of lax (15%) 6, while frequency of intermediate was (85%) 85, which show divergence among the Lines. Among the awn barbs found are smooth which have (87.5%) frequency 35, intermediate (12.5%) frequency 5, and rough are not present in any landrace of barley. The glume with awn length compare kernel length which, length of the glumes and awn shorter then kernel which present in(40%), length of the glumes and awn long then kernel with frequency (27.5%) 27.5 and glume plus awn longer then kernel with a frequency (12.5%) 12.5 which show variation among these Lines. A wide range of variability found in lemma type, on lemma have no teeth was found with frequency (22.5%) 22.5 and lemma with teeth with frequency (77.5%) 77.5and lemma with hairs were not found in any germplasm. The lemma color were observe, the normal (green color) with frequency (95%) 95, red color frequency had (2.5%) 2.5, and purple color with frequency (2.5%) 2.5 and black color were not observe. In case of awn color the white color found with frequency (17.5%) 17.5, the yellow color with frequency(77.5%)77.5, reddish color had (5%) 5, and brown color were not found an any landrace. The length of rechilla hairs was recorded which are of two types, long hair with frequency (52.5%) 52.5and short with frequency (47.5%) 47.5. There were three type color on the base of auricle pigmentation, which were observed in these Lines, the dark purple color were not found, the green color was found with frequency (90%) 90, pale purple with frequency (7.5%) 7.5, and the purple color had 2.5 with percentage (2.5%).the very low photoperiod sensitivity (2.5%),2.5 with low sensitivity (7.5%) and 7.5 had intermediate sensitivity(92%), and the high sensitive were not found and any line. In case of grain color there were no variation, the total had white color (100%). There were three type of stem pigmentation, 12.5 with green color (12.5%), 12.5 with purple color (80%) and 80 of them was with purple color (half or more) 7.5%. The lemma awns show no variation all 104 Lines had awned mean awn attached with lemma (100%). The kernel covering of total germplasm had covered there is no variation (Table 1).

Table 1. Frequency distribution of 13 qualitative morphological traits of the studied Lines.

Characters	Categories	Frequency	Percentage
Spike density	Lax	16	15
	Intermediate	14	85
Awn barbs	Smooth	87.5	87.5
	Intermediate	12.5	12.5
Glumes awns	Length of the glumes and awn shorter than kernel	40	40
	Length of the glumes and awn long than kernel	27.5	27.5
	Glume plus awn longer than kernel	12.5	12.5
Lemma type	No lemma teeth	22.5	22.5
	Lemma teeth	77.5	77.5
	Lemma hairs	0	0
Awn color	Amber/ white	17.5	17.5
	Yellow	77.5	77.5
	Brown	0	0
	Reddish	5	5
	Black	0	0
Length of Rachilla	Long	52.5	52.5
	Short	47.5	47.5
Lemma color	Normal	95	95
	Red	2.5	2.5
	Purple	2.5	2.5
	Black	0	0
Auricle pigmentation	Green	90	90
	Pale purple	7.5	7.5
	Purple	2.5	2.5
	Dark purple	0	0
Photoperiod sensitivity	Very-low	2.5	2.5
	Low	7.5	7.5
	Intermediate	92.5	92.5
	High	0	0
Grain color	White	100	100
	Purple	0	0
	Red	0	0
	Black	0	0

Stem pigmentation	Green	12.5	12.5
	Purple(basal only)	80	80
	Purple(half or more)	7.5	7.5
Lemma awns	awn less	0	0
	Awnleted	0	0
	Awned	100	100
Kernel covering	Naked grain	0	0
	Semi covered grain	0	0
	Covered grain	100	100

Quantitative parameters

There is variation noted between these landraces, which is an range of 19 to 24 days, days to germination were further divided into three categories, low (19 to 20days), intermediate (21 to 22 days), high (23 to 24 days), the mean value is 21.86 and sample variance 1.74 (Table 2), Maximum number of Lines have (37%) germinated in 38 days, while minimum no, of Lines (17%) germinated in 18 days, and (46%) are intermediate. A total of 104 landraces were studied. The range of spike length in this study divided the landraces in to three categories, low (range from 12-15 cm), medium (range 16-19cm) and high (range from 20-22 cm) with mean value as 18.17 and sample variance 4.53 (Table 2). Maximum no. of Lines had medium spike length (67%) followed by low spike length (8%) and high spike length (25%). During the present investigation a wide range of variation were noted among the 104 gemrplasms, which is an range of 24 to 32 days, days to leaf emergence were further divided into three categories, Low (24 to 32 days), intermediate (27 to 29 days), and High (30 to 32 days). the mean value is 27.56 and sample variance 3.06 (Table 2), Maximum number of Lines have (52%) 54 days for leaf emergence were found, while minimum number of Lines (32%) 33 days was found in these 104 Lines and (16%) are intermediate. In case of plant height there is a wide range of variability found among the Lines. The range of plant height between 70 to 128cm on the base of plant height divided into three categories, Low (70 to 88cm), Medium (89 to 115cm) and High (116 to 128cm), with mean value as 101.34 (Table 2) and sample variance99.22. Maximum plant height was 128cm (9%), minimum plant height was 70 (6%) and the 86% are medium plant height (Table 2). Days spike emergence in 104 lines are divided into three categories, low (117 to 120), intermediate (121 to 124) and High (125 to 128).The mean value is 121.88 and sample variance 12.61 (Table 2). Maximum days to spike emergence are 128 which is (31%) while minimum number of days to spike emergence was 117 which is 47% and 22% are intermediate.A wide range of variation was found among the Lines on the base of 100 seed weight. Range from 14g to 30g with mean value as 21.14 and sample variance 7.92 (Table 2). Maximum weight of 100 seed was 30g and minimum weight was 14g. A total of 104 Lines were divided into three categories on the basis percentage. During the present study, number of rows per spike divided into three categories, low (2 to 3rows), intermediate (4 to 5rows) and High (6 to 7rows). The range of Minimum number of rows was 2 and Maximum number was 6, the mean value is 5.94 and sample variance 0.11 (Table 2). 6 rows was found in 100 lines of *durum*.4 rows was in 3 lines while in one line there was 2 rows found. Significant variation was observed during the study on the base of biomass per plant. Which divided into three categories, range from low (10 to 33g), moderate (34 to 57g) and high (58 to 80g), the range of maximum 80g of biomass per plant and minimum 10g with mean value 29.18 and sample variance 235.61.The harvest

index also divided into three categories range from maximum 500 and minimum 34 with mean 141.33 and sample variance 6628.83 (Table 2). There is a basic variation among the breeding material of the durum wheat, like days to germination,days to leaf emergence, days to spike emergence, 100seed weight, Plant height**,** According to the net yield two groups of genotypes were selected first one are those verities which 100 seed weight was greater than 14-grame, and high yielding genotypes. While the second ones those genotypes 100- seed weight is lower than 30- gram are low yielding genotypes. The variety R-43 and R-48, have highest grain yield per plant due to higher seeds number per plant and higher grain weight while the later one had higher spikelet's per plant were observed, higher 1000grain weight and more numbers of days taken to 50% maturity. The lines R-30, R-32, R-45 and R-51, were also good yield producers due to either one or the other grain yield components. Further revealed that the numbers of seed per plant had negative genotypic correlation with the grain yield per plant is 0.126 probability level. Total Plant biomass and grain yield were highly significant**.**

Table 2. Descriptive statistics for nine quantitative character of 104 *durum* of *landraces*.

Traits	Mean	Std. Error	Std. Deviation	Sample. Variance	Minimum	Maximum	CV%
DG	21.86	0.13	1.32	1.74	20	24	6.03
DLE	27.56	0.17	1.75	3.06	24	31	6.35
DSE	121.88	0.35	3.55	12.61	117	128	2.91
Row/S	5.94	0.03	0.34	0.11	4	6	5.66
100R	21.14	0.28	2.81	7.92	14.4	30	13.32
SL	18.17	0.21	2.13	4.53	12	22	11.72
P/Biom	29.18	1.51	15.35	235.61	10	80	52.6
PH	101.34	0.98	9.96	99.22	71	127	9.83
HI%	141.33	7.98	81.42	6628.83	34.5	500	57.61

NOTE: DG= days to Germination, **DLE=** Days to Leaf emergence, **DSE=** Days to spike emergence, **Row/S=** Number of rows per spike, **100SW=** 100 seed weight, **SL=**spike length **P/biom=** Biomass per plant, **PH=** plant height, **HI=**harvest index.

The correlation among different morphological traits is given in (Table 3), the result show that some of the morphological traits were be found to be highly significance correlated with each other. Three types of them were recorded to be high values, first one is highly significant correlation which value equal or above 0.6 denoted by double star (**) like 0.637**, 0.699**, 0.445**, 0.244**, 0.469**, 0.379**, 0.928**while second one is significantly correlation, which value range from 0.00 to 1.00, like 0.07, 0.011, 0.026, 0.041,0.042, 0.062, 0.082, 0.095, 0.096, 0.156, 0.191 and 1.00. the third one is high negatively correlation which values are in highly negative like -0,330**, -0.326**, -0.434**, -0.294**, -0.303**, -0.019, -0.044, -0.109, -0.07, -0.086, -0.015, -0.157, -0.013, -0.119, Days to germination was positive strongly significant correlated to the days to leaf emergence (DLE) and (DSE) days to spike emergence and positive significant correlated to the 100 seed weight and negative significant correlated to the plant height. Days to the leaf emergence was positive strongly significant correlated to the days to spike emergence (DSE), 100 seed weight(100sw) and negative to the harvest index (HI) and negative significant correlated to plant height (PH). Days to spike emergence is strongly significant correlated to 100 seed weight and negative strongly correlated to plant biomass, plant height, harvest index. Row per spike does not shows any correlation to any characters. 100 seed weight was negative strongly correlated to harvest index. Spike length does not

show any correlation to any character. Plant biomass character was positively strongly correlated to the harvest index and the plant height was significant correlated to harvest index (Table 3).

Table 3. Correlation analysis of the quantitative traits.

Traits	DG	DLE	DSE	Row/S	100R	SP/L	P/Biom	PH	HI%
DG	1								
DLE	.637**	1							
DSE	.445**	.699**	1						
Row/S	-0.019	-0.044	0.011	1					
100SW	.244*	.469**	.379**	0.062	1				
SP/L	-0.109	-0.07	-0.086	0.095	0.041	1			
P/Biom	-0.015	-0.157	-.330**	0.096	0.007	0.042	1		
PH	-.233*	-.197*	-.326**	0.041	-0.013	0.156	0.191	1	
HI%	-0.119	-.294**	-.434**	0.082	-.303**	0.026	.928**	.223*	1

Biochemical ASSAY

SDS-PAGE was used to estimates the extent of the genetic diversity existing in the present set of the wheat genotypes. Also SDS-PAGE has one of the most widely used techniques to separates and characterized proteins. Different proteins are separated on the basis of change in their molecular weights by means of the SDS-PAGE. In this study, SDS-PAGE on the proteins of the 20 genotypes was performing by extracting protein and running on the gel in order to investigate genetic diversity. In this study, 15% polyacrylamide gel with a loading sample of 10 microliter gave the best result. Total of the 14 bands observed. By which all is polymorphic. The data was recorded on the basis of the presence and absence of protein bands, i.e.1 for the presence and 0 for the absence of the bands In the band No. 1 (B1) the frequency was 2 and the total genetic diversity was 0.90% and in band No. 2 (B2) the frequency was 8 and the total genetic diversity was 0.60%, in B3 the frequency was 12 and the total genetic diversity was 0.40%, in B4 the frequency was 10 and the total genetic diversity was 0.50%, in B5 the frequency was 7 and the total genetic diversity was 0.65%, in B6 the frequency was 9 and the total genetic diversity was 0.55%, in B7 the frequency was 8 and the total genetic diversity was 0.60%, in the B8 the frequency was 11 and the total genetic diversity was 0.45%, in B9 the frequency was 14 and the total genetic diversity was 0.30%, in B10 the frequency was 16 and the total genetic diversity was 0.20%, in B11 the frequency was 15and the total genetic diversity was 0.25%, in B12 the frequency was 7 and the total genetic diversity was 0.65%, in B13 the frequency was 4 and the total genetic diversity was 0.80% and the B14 the frequency was 16 and the total genetic diversity was 0.20%. The high genetic diversity was found in B1 which was 0.90%, the least genetic diversity was found in B14 which was 0.20% and the average total genetic diversity was 0.50% which is showing in (Table 4).

Table 4: Total genetic diversity percentage

Bands	F	P%	A%	TGD%
B-1	2	10%	90%	0.90%
B-2	8	40%	60%	0.60%
B-3	12	60%	40%	0.40%
B-4	10	50%	50%	0.50%
B-5	7	35%	65%	0.65%

B-6	9	45%	55%	0.55%
B-7	8	40%	60%	0.60%
B-8	11	55%	45%	0.45%
B-9	14	70%	30%	0.30%
B-10	16	80%	20%	0.20%
B-11	15	75%	25%	0.25%
B-12	7	35%	65%	0.65%
B-13	4	20%	80%	0.80%
B-14	16	80%	20%	0.20%
Average				**0.50%**

Cluster analysis of agro morphological character

Cluster analysis of 104 landracess for agro-morphological characters was conducted using Ward's Method. The dendogram divided into two linkage (L1 & L2) the linkage 1 (L1) further divided into 2 clusters (C1 & C2), C1 contain 4 landraces, C2 contain 6 landraces, linkage 2 further divided to 2 clusters (C3 & C4), C3 have 49 landraces, C4 have 45 landraces (Fig 1). The (Dendogram) draw for 20 wheat genotypes which are related to two main linkage distance at 50%. The linkage one contain three cluster C1, C2 &C3, the cluster 1 contain 4 landraces i.e R1, R6, R9, and R2. Cluster 2 contain 6 landraces i.e. R03 R04 R08 R018 R014 R19. Cluster 3 contain only 1 landrace of wheat R5. The linkage 2 also have 3 clusters C4, C5 & C6 the cluster C4 contain 2 landraces R7 & R11, cluster C5 contain three landraces R10, R12, R16 and cluster C6 contain also 3 landraces R13 R15, R17, R20. Sodium dodecyl sulphate polyacrylamides Gel electrophoresis is a reliable, easy and relatively quick procedure that has been widely used for the estimation of genetic variability in the crop of the commercial importance like wheat. In the present study considerable inter and intra-specific variation were observed. The bands of the wheat genotypes show the major bands categories on the basis of the glance (sharpness). The result from SDS-PAGE analysis of the wheat endosperm proteins indicates different banding pattern for different wheat genotypes and the overall degree of the variation is low, similar result of the study of the 20 genotypes of wheat were reported. Electrophoregrame showing proteins banding pattern of the different wheat verities, the result recorded from the figure show that the total numbers of the bands is 14 whose is the best result. The cluster analysis of the wheat grains glutanine proteins was performed on the result of the SDS-PAGE using the software of the statistic PC-ORD to find out the diversity among the given wheat verities. The result of the cluster analysis is given in the dendogram (Fig 2) on the basis of the linkage distance (Euclidean distance).

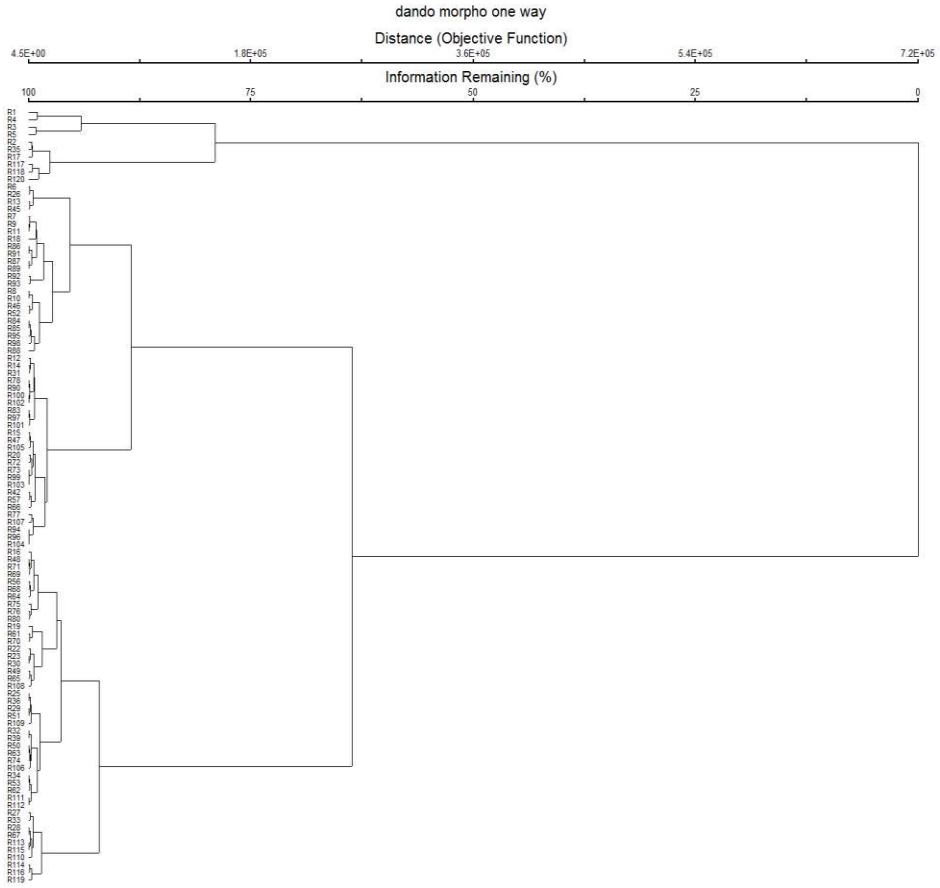

Figure 1: Morphological quantitative cluster analysis of 104 landraces used PC-ORD.

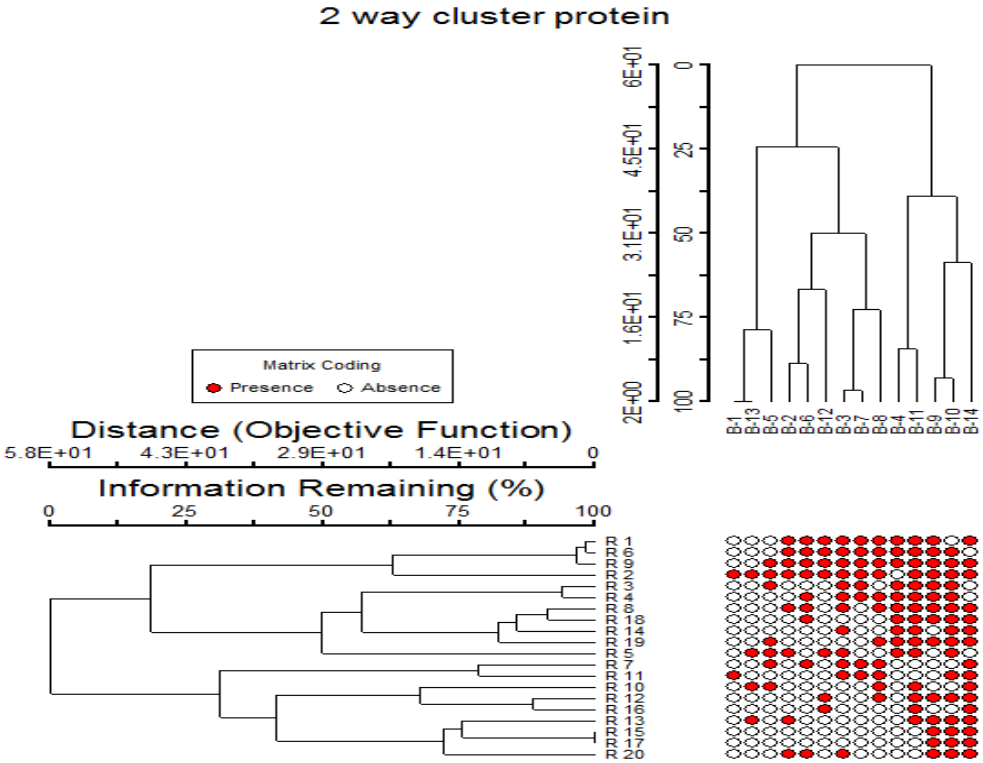

Figure 2: Dendogram for 2 way protein

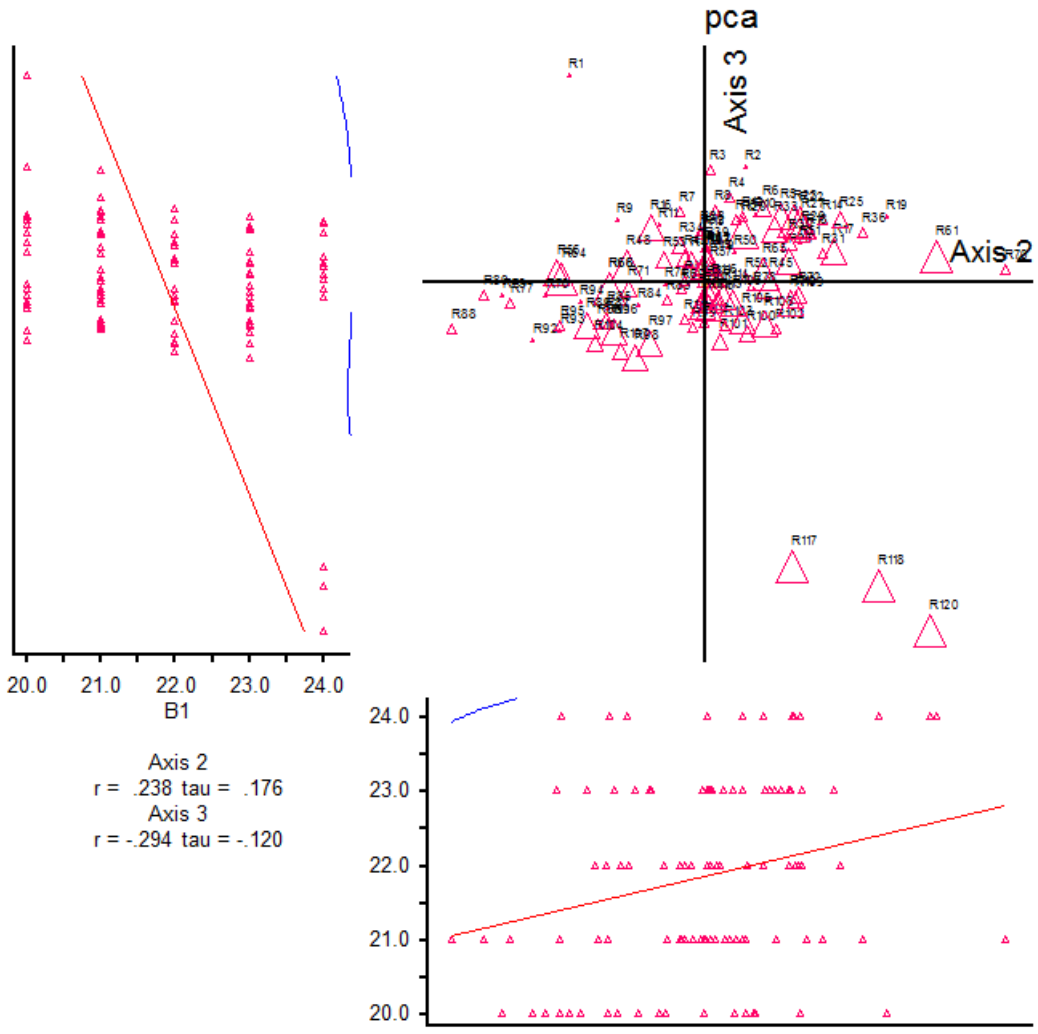

Figure 3: PCA for quantitative morphological data

Biochemical analysis

In order to estimate the genetic diversity on the base of the total seed storage protein, the band was categorized on the base of the molecular weight, and divided into two zone, I.e. from 120 KD to 180 KD show higher molecular weight, and from 40 KD to 120 KD show lower molecular weight in Fig 3.

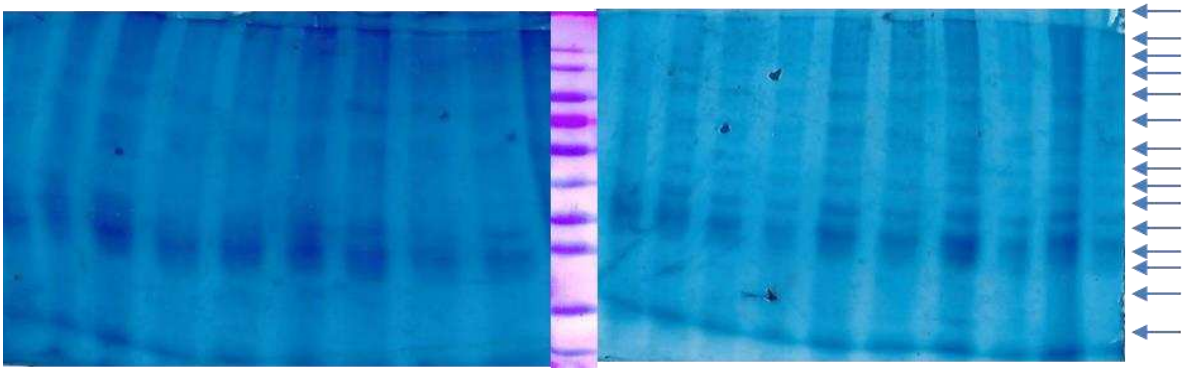

Fig 2.2: Electrophoregram showing the protein banding pattern of 20 different *Triticum durum* landraces

Discussion

The present research study show the result of different morphological & biochemical characteristic of *Triticum Durum* which collected from different areas. All the selected verities these are the unknown varieties of Malakand division.

The genotypic sequence of the Table no 2 represents that there is a basic variation among the breeding material of the durum wheat, like days to germination, days to leaf emergence, days to spike emergence, 100 seed weight, Plant height, Total Plant biomass and grain yield were highly significant. The variety R-43 and R-48, have highest grain yield per plant due to higher seeds number per plant and higher grain weight while the later one had higher spikelet's per plant were observed, higher 1000 grain weight and more numbers of days taken to 50% maturity. The lines R-30, R-32, R-45 and R-51, were also good yield producers due to either one or the other grain yield components. The Table no 3 further revealed that the numbers of seed per plant had negative genotypic correlation with the grain yield per plant is 0.126 probability level.

Correspondingly the results have been reported earlier in the form of many tables, during the present study the, inter and intra-specific variation on the basis of the Morphological traits of 104 genotypes of the durum wheat was carried out. Total 22 traits were scored, out of which 09 were quantitative and 13 were qualitative.

The cluster analysis of the 22 Morphological characters were performed using the computer software PC-ORD. the result of cluster analysis is presented as phylogenetic tree (dendrogram) in Fig 1, the phylogenetic tree divided 104 genotypes into two lineage at linkage distance 25%. The linkage-1 is further divided into three clusters at the distance of the 75%. The cluster-1 contains thirteen accessions, i.eR-1, R-3, R-33, R-21, R-5, R-15, R-50, R-7, R-23, R-2, R-27, R-11, and R-58. Cluster 2 contains ten accessions R-16, R-26, R-20, R-25, R-39, R-51, R-31, R-19, R-29, and R-34. Cluster 3 contains eight accessions i.e. R-13, R-56, R-14, R-18, R-54, R-22, R-36, and R-24. Linkage-2 (L-2) contain two clusters, cluster-4 and 5. Cluster-4 contains 16 accessions R-4, R-6, R-8, R-9, R-10, R-42, R-45, R-53, R-55, R-12, R-57, R-17, R-35, R-41, R-38, and R-49. Cluster-5 contains thirteen accessions i.e. R-28, R-30, R-32, R-37, R-60, R-40, R-47, R-43, R-44, R-46, R-59, R-48, and R-52. The scattered plot which is also draws for the confirmation of the phylogenetic relationship, through computer software PC-ORD which is also 60/ genotypes of the durum wheat into the basis of the verities differentiation.

The correlation among different morphological traits is given in Table no 3, the result show that some of the morphological traits were be found to be highly significance correlated with each other. Three types of them were recorded to be high values, first one is highly significant correlation which value equal or above 0.6 denoted by double star (**) like 0.637**, 0.699**, 0.445**, 0.244**, 0.469**, 0.379**, 0.928**while second one is significantly correlation, which value range from 0.00 to 1.00, like 0.07, 0.011, 0.026, 0.041,0.042, 0.062, 0.082, 0.095, 0.096, 0.156, 0.191 and 1.00. the third one is high negatively correlation which values are in highly negative like -0,330**, -0.326**, -0.434**, -0.294**, -0.303**, -0.019, -0.044, -0.109, -0.07, -0.086, -0.015, -0.157, -0.013, -0.119

Days to germination was positive strongly significant correlated to the days to leaf emergence (DLE) and (DSE) days to spike emergence and positive significant correlated to the 100 seed weight and negative significant correlated to the plant height. Days to the leaf emergence was positive strongly significant correlated to the days to spike emergence (DSE), 100 seed weight(100sw) and negative to the harvest index (HI) and negative significant correlated to plant height (PH). Days to spike emergence is strongly significant correlated to 100 seed weight and negative strongly correlated to plant biomass, plant height, harvest index. Row per spike does not shows any correlation to any characters. 100 seed weight was negative strongly correlated to harvest index. Spike length does not

show any correlation to any character. Plant biomass character was positively strongly correlated to the harvest index and the plant height was significant correlated to harvest index.

According to the net yield two groups of genotypes were selected first one are those verities which 100 seed weight was greater than 14-grame, and high yielding genotypes. While the second ones those genotypes 100- seed weight is lower than 30- gram are low yielding genotypes.The verities R43, R48 have high grain yield per plant due to higher grain weight the later ones whose have higher spikelet per plant, higher 100-grain weight and more number of days to 50% taken to maturity. Also the genotypes R30, R32, R45, R51 were also good yield producers due to either one or other grain yield component.

Sodium dodecyl sulphate polyacrylamides Gel electrophoresis (SDS-PAGE) is a reliable, easy and relatively quick procedure that has been widely used for the estimation of genetic variability in the crop of the commercial importance like wheat. In the present study considerable inter and intra-specific variation were observed. The bands of the wheat genotypes show the major bands categories on the basis of the glance (sharpness). The result from SDS-PAGE analysis of the wheat endosperm proteins indicates different banding pattern for different wheat genotypes and the overall degree of the variation is low, similar result of the study of the 20/ genotypes of wheat were reported. Electrophoregrame showing proteins banding pattern of the different wheat verities, the result recorded from the figure show that the total numbers of the bands is 14 whose is the best result. The cluster analysis of the wheat grains glutanine proteins was performed on the result of the SDS-PAGE using the software of the statistic PC-ORD to find out the diversity among the given wheat verities. The result of the cluster analysis is given in the dendrogram (Fig no 2) on the basis of the linkage distance (Euclidean distance).

SDS-PAGE was used to estimates the extent of the genetic diversity existing in the present set of the wheat genotypes. Also SDS-PAGE has one of the most widely used techniques to separates and characterized proteins. Different proteins are separated on the basis of change in their molecular weights by means of the SDS-PAGE. In this study, SDS-PAGE on the proteins of the 20 genotypes was performing by extracting protein and running on the gel in order to investigate genetic diversity. In this study, 15% polyacrylamide gel with a loading sample of 10 microliter gave the best result. Total of the 14 bands observed. By which all is polymorphic. The data was recorded on the basis of the presence and absence of protein bands, to find out the genetic diversity among durum wheat genotypes cluster analysis was carried out. The result of the cluster analysis is presented as phylogenetic tree (Dendrogram) in Fig 2. The (Dendrogram) draw for 20 wheat genotypes which are related to two main linkage distance at 50%. The linkage one contain three cluster C1, C2 &C3, the cluster 1 contain 4 landraces i.e R1, R6, R9, and R2.Cluster 2 contain 6 landraces i.e R03 R04 R08 R018 R014 R19. Cluster 3 contain only 1 landrace of wheat R5. The linkage 2 also have 3 clusters C4, C5 & C6 the cluster C4 contain 2 landraces R7 & R11, cluster C5 contain three landraces R10, R12, R16 and cluster C6 contain also 3 landraces R13 R15, R17, R20

Similarly scattered plot (Fig 2) for the molecular data were also draw for the confirmation of the phylogenetic tree based on SDS-PAGE. The scattered plot were also groups of all genotypes into four clusters similar to that of two way cluster analysis. All of these indicate that SDS-PAGE could group the genotypes on the basis of intra and inter-specific variation in protein profile with thin and among verities.

Conclusion

Briefly our data showing significant variation in the Morphological characters analysis and SDS-PAGE analysis among durum wheat varieties which is from Swat and Shangla, this study related by using wheat biochemical markers revealed considerable amounts of the Genetic diversity among 104

wheat verities that can be used in selecting diverse parent in breeding program and maintaining genetic variation in the landraces of the *Triticum durum.*The maximum genetic diversity was found 0.90%, while the minimum genetic diversity was found 0.20% and the average total genetic diversity was 0.50%.

Further assessment of these lines are needed on Gene Mining to manipulate Novel alleles from selected lines of Durum.

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