INNSPUB

International Journal of Biosciences | IJB |
ISSN: 2220-6655 (Print) 2222-5234 (Online)
http://www.innspub.net
Vol. 25, No. 6, p. 468-484, 2024

RESEARCH PAPER OPEN ACCESS

Molecular analysis of drought-resistant cultivars in selected wheat genotypes

Mustafa Kamal[1], Shafee Ur Rehman[*2]

[1]*Department of Biotechnology and Genetic Engineering, Hazara University, Mansehra, Khyber Pakhtunkhwa, Pakistan*

[2]*Faculty of Medicine, Ala-Too International University, Ankara, Bishkek, Kyrgyzstan*

Key words: Wheat, Drought, Scot and polymorphism

http://dx.doi.org/10.12692/ijb/25.6.468-484 Article published on December 11, 2024

Abstract

Climate changes and global warming have seriously affected the agriculture industry worldwide. Decrease in water has significantly couse reduction in production and yield of many crops. Wheat is a major cereal crop cultivated around the globe. The current research was conducted to investigate drought resistant cultivars in the selected wheat cultivars. In the present study fifty wheat cultivars, scot markers were used during the experiment. The experiment was planned in Randomized Complete Block Design (RCBD) with 3 replications. The analysis of variance showed highly significant differences among the genotypes. The maximum mean value of plant height, tiller per plant, flag leaf area, spike length, spikelet's per spike, grain per spike, biological yields, Yield/plant, 1000 grains weight, harvest index, spikes density, yield per hectare and grain per spike were found in genotypes g10, g31, g35, g12, g28, g49 g20, g20, g3, g47, g39, g20, g46. The genotype g20 showed better result towards biological yield, yield per plant, and yield per hectare that is related with yield associated traits. Scot markers are practiced for 50 wheat genotypes. Such as scot 7, scot 10, scot 13, and scot18 show high level (100%) of polymorphism. No such marker produced monomorphic bands.

* **Corresponding Author:** Shafee Ur Rehman ✉ shafeeur.rehman@alatoo.edu.kg

Introduction

In the early 1960s improved conventional wheat breeding adopted with better cultural practices led to a generous wheat production in the world known as "green revolution". According to (Dixon, 2009), wheat demand is increasing faster and it is expected that will be achieve to 40 %percent in one decade. Therefore, it is necessary to enhance wheat yield to sustainable food security. Several problems exist which are accountable for lower wheat production, as well as low quality of seed, using improper broadcasting methods for sowing, late cultivation, worst soil, uneven fertilizer doses, unsuitable weed eradicating, disease and less supply of water and heat as the results of climate changes (Ahmed *et al.,* 2019a).

Among cereals crops, wheat crop status is important due to the nutritional values and more feeding. Massive growth in populations and the liberated life style has directed to emerging issues/ problems for wheat scientists to create new genotypes having prominent yield in water deficit areas and improved quality seed (Mujtaba *et al.,* 2016). Crop productivity faces many challenges, one of them the biggest challenge is drought, which is mainly due to changes in the pattern of precipitation and inadequate rainfall pattern (Faisal *et al.,* 2017). Different mechanisms involved in plant body to manage the drought stress. In the previous years, numerous restrictions stuck about the drought mechanisms because poor concept of developmental and physiological basis for yield contribution attributes under drought environments due to polygenic inheritance pattern of drought tolerance associated characters (Khan *et al.,* 2018). Drought stress is a complex mechanism so, number of genes/QTLs having trivial effects which regulate this mechanism (Ahmed *et al.,* 2017a). To create tolerance against water deficit, it is very important to primarily understand the mechanism and behavior of the plant under drought stress conditions. To stand against the water shortage conditions plant has various developmental phases like, morphologically, physiologically, biochemically, anatomically and at molecular level. Drought tolerance mechanism is complex at a cellular and molecular level as well as whole plant body level.

Several details contribute concern the complication of drought tolerant system like, crops species, intensities, and duration of stresses and plants development stages (Saeidi and Abdoli, 2018). Survivals of plants in drought may be adoptive several tolerance mechanisms operative simultaneously. Mainly three basics mechanism, a plant can familiarize to face in water deficit conditions. (i) Escape (ii) avoidance or tolerance (iii) resistance mechanism. In first mechanism, plant completes the life cycles before shortage of water. In second mechanism, plant take step to face in the condition of less supply of water, e.g. close of stomatal opening, decrease the rates of transpirations. In third mechanism, plants take step at the cell levels against water deficit conditions through developing antioxidant which maintain of osmotic adjustments and at tissue level (Wang *et al.,* 2013; Shahinnia *et al.,* 2016).

Various morphological modifications occur in plants body under drought environment like leaf area shrinkage, stomata frequency reduce, cell walls thickening of leaf, epicuticles waxes deposition, and conductive system development, large vessels frequency increase, senescence before maturity and formation of leaves in cereals like tubes structure (Ahmed *et al.,* 2017b). Drought is also occurring due to high temperature of climate, which disturbs the photosynthesis process by raising the evapotranspiration rate. Among different abiotic stresses restricting the productivity of crop, the most difficult and complex to breeding is drought due to polygenic in nature (Zhu *et al.,* 2016).

Plant and water relation are badly affected by drought stress, resultantly total water contents reduced and altered the turgor of cells. It also induces stomata closure, reduces the rate of transpiration, restrictions in gases exchanges and inhibits photosynthetic

activity (Kosar *et al.,* 2015). Due to drought stress, several structure and function alterations in photosynthesis machineries such as modifications in the photosynthetic pigments (chlorophyll a & b), slow down the CO_2 uptake process due to closure of stomata and shortage of photosynthates assimilation because inhibit the chloroplast activity (Liu *et al.,* 2016). One of the main causes of photosynthetic suppression is the formations of (ROS) like super oxides and hydroxyl radicals, which impaired the photosynthetic machinery. Under water deficient condition the synthesis of chlorophyll is inhibited.

Minerals uptake and transport process are badly affected and ultimately reduced the leaf area, changed assimilate partitioning and finally decrease the yield of wheat plant. Wheat production decreased from 50 to 90 % of their irrigation potentials in the progressing areas by drought (Li *et al.,* 2017). In plants cell, photosynthetic is an important mechanism and regulate under low concentration of water culture medium. If chlorophylls pigment concentrations increase, then photosynthetic systems will be great effectual. The chlorophylls content causes greater decrease in wheat with increased amount of drought, as the thylakoids membrane disintegrates with cell dehydrations (Maghsoudi *et al.,* 2015). The amount of leaf chlorophyll contents is the indicator of the photosynthetic capacity of plant tissue. Decreased or unchanged levels of chlorophylls in drought conditions previously, stated in several crops, as of water shortage occurred. The amount of chlorophyll content changes in cereal crops especially in wheat under water deficit environments (Barutçular *et al.,* 2016). The choice of wheat accessions based on the characteristics of the seedlings is informal, low-priced and less hassle. Likewise, the seedlings attributes were exhibit moderates to high variation with an additives genetics effects on the environments (Rehman *et al.,* 2016). The current experiment was performed for the selection of fifty different wheat accessions for drought tolerance on the base of evaluation of the selected wheat lines for high yield under drought condition of Mansehra, and molecular characterization of the selected lines using Scot markers.

Materials and methods

The current study was conducted under field condition of Mansehra in 2017-2018. Fifty wheat drought tolerant genotypes were collected for genetic analysis and for morphological traits.

Table 1. Detail of selected wheat genotypes for the current study

SL	Genotype	Source	Genotype codes
1	Local CHECK	Local cross	g1
2	SOKOLL/WBLL1 PTSS02Y00021S-099B-099Y-030ZTM-040SY-040M-18Y-0M-OSY-0B-0Y	CIMMYT	g2
3	SOKOLL/WBLL1 PTSS02Y00021S-099B-099Y-030ZTM-040SY-040M-9Y-0M-OSY-0B-0Y	CIMMYT	g3
4	PASTOR//HXL7573/2*BAU/3/WBLL1	CIMMYT	g4
5	SOKOLL//W15.92/WBLL1	CIMMYT	g5
6	FRTL//ATTILA/3*BCN	CIMMYT	g6
7	OR791432/VEE#3 .2//ATTILA/3*BCN	CIMMYT	g7
8	PUB94.15.1.12/WBLL1	CIMMYT	g8
9	MEX94.27.1.20/3/SOKOLL//ATTILA/3*BCN	CIMMYT	g9
10	SOKOLL/WBLL1 PTSS02Y00021S-099B-099Y-099B-099Y-43B-0Y	CIMMYT	g10
11	SOKOLL/WBLL1 PTSS02Y00021S-099B-099Y-030ZTM-040SY-040M-5Y-0M-OSY-0B-0Y	CIMMYT	g11
12	SOKOLL/WBLL1 PTSS02Y00021S-099B-099Y-030ZTM-040SY-040M-21Y-0M-OSY-0B-0Y	CIMMYT	g12
13	WBLL4//OAX93.24.35/WBLL1 PTSS02B00110T-0TOPY-0B-0Y-0B-24Y-0M-OSY-0Y-0Y	CIMMYT	g13
14	CN079//PF70354/MUS/3 PASTOR/4/BAV92/5/FRET2/KUKUNA//...CMSA05Y01011T-040M-040ZTM-040SY-4ZTM-03Y-0B	CIMMYT	g14

15	PFAU/MILAN/S/CHEN/AEGIL OPS SQUARROSA (TAUS)//BCN/3/...CMSS02Y00613S-59Y-0M-0WGY-0B	CIMMYT	g15
16	SLVS//ATTILA*2/M10 (MUTATED C-306) CMSA00164S-040P0M-13CRE-010M-010SY-7M-0Y-0SY-0Y	CIMMYT	g16
17	SOKOLL*2/TROST SMSA05Y01186T-040M-040ZTPOY-040ZTM-040SY-32ZTM-02Y-0B	CIMMYT	g17
18	SOKOLL/ROLFO7 SMSA04M00346S-040ZTPOY-040ZTM-040SY-28ZTM-01Y-0B	CIMMYT	g18
19	SW94.2690/SUNCO SMSA01M00069S-040P0M-030ZTM-040SY-28ZTM-040SY-040M-1Y-0M-OSY-0Y-0Y	CIMMYT	g19
20	WHEAR//2*PRL/2*PASTOR CGSS03B00090T-099Y-099Y-099M-099Y-099M-17WGY-0B	CIMMYT	g20
21	CHEN/AE.SQ//2*OPATA/3FINSI CMSA00M00128S	CIMMYT	g21
22	W15.92/4/PASTOR//HXL7573/2*BAU/3/WBLL1 PTSS02B00102T-0TOPY-0B-0Y-0B-11Y-0M-OSY-0B-0Y	CIMMYT	g22
23	SOKOLL/WBLL1 PTSS02Y00021S-099B-099Y-234B-0Y	CIMMYT	g23
24	HGO94.9.1.37/2*NAVJO7 PTSA08M0008T-050Y-050ZTM-050Y-3ZTM-010Y-0B	CIMMYT	g24
25	CHEN/AE.SQ//2*WEAVER//3/BAV92	CIMMYT	g25
26	Pastor//HXL7573/2*BAU/3/ATTILA/3*BCN/4/SOKOLL/...	CIMMYT	g26
27	SOKOLL/3/PASTOR//HXL7573/2*BAU/3/ATTILA/PASTOR	CIMMYT	g27
28	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/SRMA/TUI...	CIMMYT	g28
29	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/PARAS/PASTOR	CIMMYT	g29
30	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/PARAS/PASTOR	CIMMYT	g30
31	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/ASTRES	CIMMYT	g31
32	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/MEX94.2.19//SOKOLL/...	CIMMYT	g32
33	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/MEX94.2.19//SOKOLL/...	CIMMYT	g33
34	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/WBLL4//OAX93.24.35/...	CIMMYT	g34
35	SOKOLL/3/PASTOR//HXL7573/2*BAU/S/CROC-1/...	CIMMYT	g35
36	PASTOR//HXL7573/2*BAU/3/ATTILA/3*BCN/4/SOKOLL/3/PASTOR/...	CIMMYT	g36
37	SOKOLL/3/PASTOR//HXL7573/2*BAU/4/PARAS/PASTOR/...	CIMMYT	g37
38	WBLL4//OAX93.24.35/WBLL1/5/CROC-1/AE.SQUARROSA (205)//...	CIMMYT	g38
39	PUB94.15.1.12/RTL//92.001E7.7.32.5/SLVS	CIMMYT	g39
40	SOKOLL	CIMMYT	g40
41	ROELEFS 2007	CIMMYT	g41
42	WEEBILL1	CIMMYT	g42
43	KACHU #1	CIMMYT	g43
44	BAJ #1	CIMMYT	g44
45	REESDLING #1	CIMMYT	g45
46	Sariab 92	Local	g46
47	Sarab 73	Local	g47
48	C228	Local	g48
49	Meraj 8	Local	g49
50	Chakwal 86	Local	g50

Morphological studies

Fifty wheat genotypes were planted in favourable seeding time with 3 replications in a randomized complete block design, recommended agriculture practices were done for sowing wheat (Table 1). Spacing were kept as 30cm between the rows. The data was randomly collected from three selected plants in each row for parameters i.e. plant height, tillers/plant, flag leaf area, spikelet's/spike, spike length, biological yield, harvest index, yield per plant, grain/spike etc. The data were recorded on the following parameters.

Plant height (cm)

At maturity three plants selected arbitrarily were measured from base of plants to the base of spike. Height of plant was measured with meter rod in cm.

Tillers per plant

Three plants were randomly selected from each treatment to determine productive tillers/plant.

Flag leaf area

For measuring leaves of main shoot, the maximum breadth and length was measured (cm^2). Data

recording was when the leaves were fully turgid. Formula used is:

Flag leaf area= length × width × 0.74.

Spike length (cm)

Spike length from selected main shoot was measured in centimetres from base of the spike to its tip without awns using three randomly selected spikes without awns.

Spikelet's per spike

The spikelet number was counted from the main spike of selected guarded plants.

Grain per spikelet

Grain per spikelet were counted for each spikelet of a spike and then there mean value was taken.

Biological yield

The 3 randomly plants from each row was harvested and weighted by electronic balance record the biological yield.

Yield per plant

From 3 replication samples were taken and weighted by electronic balance to find the average yield per plant.

1000 grain weight (g)

Samples from each replication were collected and weighted to obtain 1000 grain weight.

Harvest index

Harvest index (HI) = $\frac{Grain\ yield}{Bio\ YIELD}$ x 100

Yield per hectare

Yield per hectare was finding by the following formula:

Area covered by one plant= Area covered by plants under observation/Number of plants under observation

Plants in one hectare = 10,000m²(hectare)/Area covered by a single plant

Yield per hectare = yield per plant × plants in one hectare

Spike density

Spike density was finding by spikelet/spike divided by spike length.

Formula used for spike density was the following:

Spikes density = $\frac{\text{Spikelet/spike}}{\text{Spike lenght}}$

Grain/Spike

Total grain of the spikes used for grain per spike was weighted to note the average grain weight per spike.

Molecular study

Molecular study was done for 50 wheat genotypes using scot markers.

Seed germination

Seeds were surface sterilized by putting seed into 70% ethanol for less than one minute and then wash with distilled water. The washed seeds were then put in 1% sodium hypo chloride for 15 minutes and washed 3 times with distilled water. The soft seeds were put on moist filter paper in Petri dishes and placed for forty eight hours. The leaves and roots were arising. The fresh leaves were then used for DNA isolation process (Fig. 1).

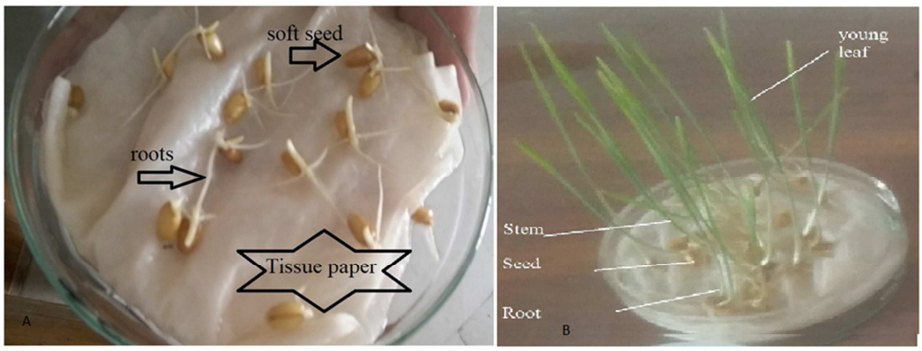

Fig. 1. a) Germination of seeds in functional genomics lab, b) Fresh young leaves of wheat used for molecular study

Genomic DNA isolation

Genomic DNA from particular plants was extracted using weining and langridge (1992) procedure. Collected 10cm leaf sample were put in eppendorf tube and poured 500µl DNA extraction buffer into eppendorf tube containing leaves. The selected samples were broken with needle. 400 µL of phenol: Chloroform: isoamyl alcohol (in the ratio of 25:24:1) were added and vortexed. The centrifuging was carried out at 8000 rpm for 15minutes. Supernatant was collected in a separate eppendorf tube and 50µl of 3M sodium acetate and 500µl Iso propanol was added. The processes of centrifugation with 8000rpm for 10minutes were done to get the DNA pellet. The pellet then washed with 70 % ethanol to remove the unwanted material and kept it for overnight to dry. Distilled water or TE buffer was added to the dry pellet and was mixed will and kept at 4°C. 1% agarose gel was used to check the quality and quantity of the genomic DNA. The loaded gel was run at 70 volts for 35 minutes. Gel was checked under UV light using UVTECH System.

In current research work scot markers were used (Table 2). The markers were further confirmed by PCR and gel photograph was taken for further analysis (Fig. 2).

Table 2. Primers name, their sequences and Size of the product

SL	Names	Sequence	bp range
1	Scot 2	CAACAATGGCTACCACCG	200-1500
2	Scot 3	AAGCAATGGCTACCACCA	500-1500
3	Scot 7	CATGGCTACCACCGGCCC	450-550
4	Scot 10	CAACAATGGCTACCAGCC	200-700
5	Scot 13	ACGACATGGCGACCATCG	500-1000
6	Scot 18	CATGGCTACCACCGGCCC	200-400

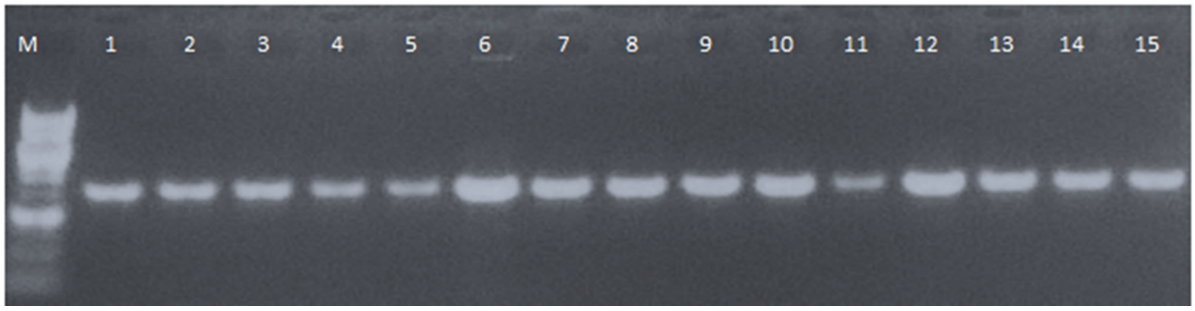

Fig. 2. Extracted DNA from the wheat genotypes

Results

Collected data were subjected to the analyses of variance (ANOVA) followed by Least Significant Difference (LSD) test to compare the differences among means of treatments (Steel *et al.*, 1997)(Table 3, 4, 5, 6 and 7).

Plant height

The analysis of variance (ANOVA) was found extremely significant between all the genotypes. The maximum plant height was found in genotype g10(115.77cm) followed by g6(111.80cm) and g3(103.83cm) while the minimum plant height was recorded in genotypes g9(72.13cm).

No of tillers/plant

It is very essential yield related trait. In these selected genotypes, the analysis of variance for this trait showed very significant b/w all the genotypes. The genotypes 31(8.0000), 37(7.3333), 40(7.0000) showed maximum number of tillers/plant while minimum number of tillers/plant were recorded in genotype 17(3.0000).

Flag leaf area

It is important because it provide food for plant. For leaf area with all the genotypes considerable variation was recorded through analysis of variance (ANOVA). The maximum flag leaf was noted in genotype g35(43.667

cm²) followed by genotype g49(42.867 cm²) and g21 (42.333 cm²) while g42 (26.333cm2) showed minimum flag leaf area.

Spike length

The analysis of variance (ANOVA) shown highest variation among the genotypes. The highest spike length were found in genotypes 12(11.700cm), 22 (11.300cm), 40(11.200cm), while the lowest Spike Length shown in genotype 16(7.867cm).

Spikelet's per spike

The genotype g28(19.000) showed maximum no. of spikelets/spike followed by g10(18.667), g40(18.667), while minimum no. of spikelets/spike were recorded in g45(13.000).

Table 3. Analysis of variance for 13 studied parameters

SL	Traits	Replication	Genotypes	Error	Probability
1	Plant height	158.241	156.152	112.945	0.0880
2	No of tiller per plant	3.62000	3.29537	3.19823	0.4411
3	Flag leaf area	589.988	69.331	53.742	0.1431
4	Spike length	2.24000	2.43308	1.10769	0.0005
5	Spikelet's per spike	8.18667	5.81782	2.66966	0.0005
6	Grain per spikelet's	0.32000	0.75769	0.53769	0.0760
7	Biological yield	56.4424	81.5832	66.1024	0.1883
8	Yield per plant	8.4023	12.1357	9.2516	0.1281
9	1000 grain weight	196.749	79.726	58.551	0.0985
10	Harvest index	35.996	202.650	153.483	0.1225
11	Yield per hectare	4117144	5946505	4533297	0.1281
12	Spike density	0.00454	0.04228	0.03091	0.0953
13	Grain per spike	227.947	122.942	55.641	0.0004

Table 4. Performance of wheat genotypes for morphological traits

SL	Genotypes	Parameters					
		Plant height	No of tillers	Flag leaf area	Spike length	Spikelet's/spike	Grain per spikelet
1	g1	85.83 GH	5.6667 ABCDEF	29.000 EFGHI	9.133FG H-O	16.667 ABCDEFG	3.3333 BCD
2	g2	101.73 ABCDEFG	4.6667 BCDEF	30.000 CDEFGHI	8.467 KLMNO	14.667 GHI	3.0000 BCDE
3	g3	107.83 ABC	4.6667 BCDEF	34.700 A-I	11.100 ABCD	16.667 ABCDEFG	2.6667 CDE
4	g4	98.63 ABCDEFG	5.3333 ABCDEF	39.667 ABCDEF	9.000GH IJKLMNO	15.333 EFGHI	3.0000 BCDE
5	g5	99.03 ABCDEFG	6.3333 ABCDE	30.333 CDEFGHI	10.700 ABCDEFG	18.333 ABC	2.6667 CDE
6	g6	111.80 AB	4.0000 DEF	38.333 ABCDEFGH	9.267FG HIJKLMNO	17.000 ABCDEFG	2.3333 DE
7	g7	86.80 FGH	5.0000 BCDEF	29.667 CDEFGHI	9.400 D-O	17.000 ABCDEFG	2.3333 DE
8	g8	103.10 ABCDEF	5.0000 BCDEF	38.000 A-I	10.200 A-J	16.667 ABCDEFG	2.3333 DE
9	g9	72.13H	5.6667 ABCDEF	29.000 EFGHI	11.033 ABCDE	17.667 ABCDE	2.6667 CDE
10	g10	115.77A	4.3333 CDEF	39.667 ABCDEF	10.000 A-LM	18.667 AB	3.0000 BCDE
11	g11	103.73 ABCDEF	4.3333 CDEF	36.000 A-HI	10.633 ABCDEFG	18.000 A-D	3.0000 BCDE
12	g12	102.87 ABCDEFG	3.6667 EF	40.000 ABCDE	11.700 A	17.667 ABCDE	3.0000 BCDE
13	g13	96.27 BCDEFG	3.6667 EF	30.000 CDEFGHI	10.133A-L	17.333 ABCDEF	3.0000 BCDE
14	g14	92.87 CDEFG	4.6667 BCDEF	29.000 EFGHI	10.633 ABCDEFG	17.667 ABCDE	3.3333 BCD
15	g15	98.33 BCDEFG	5.0000 BCDEF	35.333ABCDE FGHI	10.500ABCDEFG H	18.000 ABCD	3.0000 BCDE

16	g16	86.90 FGH	4.6667 BCDEF	27.667 GHI	7.867 O	14.667 GHI	3.666 7ABC
17	g17	89.00DEFGH	3.0000 F	38.333 ABCDEFGH	9.633BCDEFGHIJKLMN	16.000 CDEFGH	2.3333 DE
18	g18	93.47 CDEFG	3.6667 EF	33.333ABCDEFGHI	9.633BCDEFGHIJKLMN	17.333 ABCDEF	2.6667 CDE
19	g19	103.23ABCDEF	4.6667 BCDEF	33.000ABCDEFGHI	9.633BCDEFGHIJKLMN	19.000 A	4.0000 AB
20	g20	96.80 BCDEFG	6.3333ABCDE	34.667AB CDEFGHI	10.400 ABCDEFGHI	17.000A BCDEFG	2.6667 CDE
21	g21	98.53 BCDEFG	4.3333 CDEF	42.333 AB	10.433 ABCDEFGH	18.000 ABCD	3.3333 BCD
22	g22	100.90 ABCDEFG	6.0000 ABCDE	31.333 BCDEFGHI	11.300 AB	17.667 ABCDE	3.0000 BCDE
23	g23	99.93 ABCDEFG	5.6667 ABCDEF	39.000 ABCDEFG	10.367 ABCDEFGHI	15.333 EFGHI	3.3333 BCD
24	g24	96.87 BCDEFG	4.6667 BCDEF	31.000BC DEFGHI	10.367 ABCDEFGHI	18.000 ABCD	2.6667 CDE
25	g25	96.80 BCDEFG	5.3333 ABCDEF	37.333AB CDEFGHI	8.900 HIJKLMNO	17.000 ABCDEFG	2.3333 DE
26	g26	96.87 BCDEFG	6.6667 ABCD	36.000 ABCDEFGHI	9.633BCDE FGHIJKLMN	18.000 ABCD	3.3333 BCD
27	g27	95.40 BCDEFG	4.0000 DEF	33.000 ABCDEFGHI	9.433DEFG HIJKLMNO	15.333 EFGHI	3.3333 BCD
28	g28	95.23 BCDEFG	5.6667 ABCDEF	38.333 ABCDEFGH	10.433 ABCDEFGH	19.000 A	2.6667 CDE
29	g29	98.63AB CDEFG	3.6667 EF	35.333 ABCDEFGHI	10.167 ABCDEFGHIJK	17.000A BCDEFG	3.0000 BCDE
30	g30	97.03 BCDEFG	4.3333CDEF	30.333 CDEFGHI	9.267FGH IJKLMNO	16.333 BCDEFGH	3.0000 BCDE
31	g31	99.47AB CDEFG	8.0000 A	28.000 FGHI	8.800 HIJKLMNO	15.333 EFGHI	2.3333 DE
32	g32	94.47 CDEFG	4.3333 CDEF	35.000 ABCDEFGHI	9.500CDEFGHIJKLMNO	16.000 CDEFGH	2.6667 CDE
33	g33	96.43 BCDEFG	4.0000 DEF	27.667 GHI	9.033 GHIJKLMNO	15.000 FGHI	2.6667 CDE
34	g34	106.17 ABCD	4.3333 CDEF	41.333 ABC	10.233 ABCDEFGHIJ	16.667 ABCDEFG	3.0000 BCDE
35	g35	105.67 ABCD	4.0000 DEF	43.667 A	9.333 EFGHIJKLMNO	16.000 CDEFGH	2.3333 DE
36	g36	94.33 CDEFG	3.6667 EF	42.333 AB	8.700 IJK-O	16.333 B-H	3.3333 BCD
37	g37	92.50 CDEFG	7.3333 AB	28.667 EFGHI	8.433 LMNO	14.667 GHI	2.6667 CDE
38	g38	95.40 BCDEFG	5.0000 BCDEF	35.333 A-HI	8.300 MNO	14.000 HI	3.3333 BCD
39	g39	91.87 CDEFG	5.6667 ABCDEF	34.333 ABCDEFGHI	8.633 JKLMNO	16.667 ABCDEFG	2.3333 DE
40	g40	104.50 ABCDE	7.0000 ABC	33.000 ABCDEFGHI	11.200 ABC	18.667 AB	3.3333 BCD
41	g41	104.50 ABCDE	5.0000 BCDEF	41.000 ABCD	9.933 B-LM	15.333 EFGHI	2.6667 CDE
42	g42	96.83 BCDEFG	4.6667 BCDEF	26.333 I	9.633 B-N	16.000 CDEFGH	2.6667 CDE
43	g43	96.00 BCDEFG	3.6667 EF	37.000 ABCDEFGHI	10.300 A-J	16.667 ABCDEFG	2.0000 E
44	g44	98.27 BCDEFG	4.0000 DEF	29.333 DEFGHI	8.433 LMNO	14.667 GHI	2.6667 CDE
45	g45	93.87 CDEFG	4.3333 CDEF	36.333 ABCDEFGHI	9.500 C-O	13.000 I	3.0000 BCDE
46	g46	92.33 CDEFG	3.6667 EF	33.533 ABCDEFGHI	10.200 A-J	18.000 ABCD	3.3333 BCD
47	g47	87.33 EFGH	5.0000 BCDEF	31.000 BCDEFGHI	8.000 NO	14.000 HI	4.0000 AB
48	g48	91.67	4.3333	27.033	9.533	15.333	3.3333

		CDEFG	CDEF	HI	C-O	EFGHI	BCD
49	g49	97.67 BCDEFG	4.3333 CDEF	42.867 AB	10.800 ABCDEF	17.333 ABCDEF	4.6667 A
50	50	87.00 FGH	4.0000 DEF	40.000 ABCDE	9.733 B-M	18.333 ABC	2.6667 CDE

Table 5. Performance of wheat genotypes for morphological characters

SL	Genotype	Parameters						
		B yield	Y/plant	1000-grain weight	Harvest index	Y/hectare	Spike density	Grain/spike
1	g1	15.217 DEFGH	6.067 BCDE	42.133 FGHIJK	39.657 BCDEFGHIJ	4246.7 BCDE	1.8470 ABCDEF	41.667 AB-H
2	g2	20.44 B-H	5.967 BCDE	49.033 A-JK	30.030 IJ	4176.7 BCDE	1.7263 AB-H	43.667 A-G
3	g3	22.70 A-H	9.400 ABC	57.833 A	44.510 A-IJ	6580.0 ABC	1.5130 HI	23.667 K
4	g4	14.817 EFGH	5.100 BCDE	39.933 JK	35.007 D-J	3570.0 BCDE	1.7039 AB-GH	28.667 JK
5	g5	21.98 A-H	6.867 BCDE	46.300 A-K	30.423 HIJ	4806.7 BCDE	1.7151 ABC-GH	33.000 E-K
6	g6	16.600 DEFGH	8.433 ABCDE	46.500 A-JK	57.107 AB	5903.3 ABCDE	1.8368 AB-F	29.000 IJK
7	g7	19.533 BC-H	9.733 AB	52.167 AB-IJ	55.343 ABC	6813.0 AB	1.8175 AB-G	35.000 EF-JK
8	g8	17.500 DEFGH	8.067 ABCDE	52.333 AB-I	51.903 A-F	5646.7 A-E	1.6509 BC-HI	32.333 F-K
9	g9	19.267 BC-H	6.333 BCDE	54.767 ABCD	33.020 E-J	4433.3 BCDE	1.5969 EFGHI	37.000 D-J
10	g10	16.457 DEFGH	6.433 BCDE	50.067 A-JK	44.150 A-IJ	4503.3 BCDE	1.8672 ABCDE	45.000 A-E
11	g11	18.500 BC-H	7.000 BCDE	51.267 A-K	36.523 C-J	4900.0 BCDE	1.6896AB-GH	38.667 B-J
12	g12	20.513 B-H	6.933 BCDE	57.233 A	33.720 EF-J	4853.3 BCDE	1.5128 HI	39.000 B-IJ
13	g13	15.267 DEFGH	6.000 BCDE	45.867 A-JK	40.203 B-J	4200.0 BCDE	1.7336 AB-H	38.000 CD-IJ
14	g14	21.300 B-GH	5.533 BCDE	41.733 GHIJK	24.840 J	3873.3 BCDE	1.6757 ABC-GH	37.667 D-IJ
15	g15	20.333 BCDEFGH	8.767 ABCD	43.233 C-JK	51.583 A-G	6136.7 ABCD	1.7098 A-H	41.000 AB-HI
16	g16	11.133 H	5.200 BCDE	44.300 B-IJK	48.203 A-I	3640.0 BCDE	1.8719 A-E	47.667 ABCD
17	g17	20.600 BCDEFGH	8.067 ABCDE	40.700 IJK	36.753 C-J	5646.7 ABCDE	1.6614 A-I	36.000 DEFGHIJ
18	g18	21.067 BCDEFGH	7.733 BCDE	46.033 A-K	35.517 C-IJ	5413.3 BCDE	1.8108 A-G	47.333 ABCD
19	g19	26.367 ABCDE	8.833 ABCD	42.467 DEFGHIJK	34.157 D-J	6183.3 ABCD	1.6211 C-I	39.000 B-HIJ
20	g20	35.100 A	12.900 A	54.533 A-E	37.217 BC-J	9030.0 A	1.6302 C-HI	37.000 D-J
21	g21	23.900 A-H	8.167 ABCDE	50.200 A-K	35.443 C-J	5716.7 ABCDE	1.7299 A-H	41.667 AB-GH
22	g22	15.700 DEFGH	5.300 BCDE	50.233 A-K	32.237 FGHIJ	3710.0 BCDE	1.5630 FGHI	36.000 DE-IJ
23	g23	18.333 C-H	7.500 BCDE	47.833 A-K	41.787 A-J	5250.0 BCDE	1.4834 HI	34.333 EF-K
24	g24	31.567 AB	8.867 ABCD	47.033 AB-K	28.910 IJ	6206.7 ABCD	1.7590 A-H	39.333 B-J
25	g25	25.333 A-F	7.900 BCDE	39.667 K	32.910 EFGHIJ	5530.0 BCDE	1.9350 AB	50.00 ABC
26	g26	22.867 A-H	8.900 ABCD	49.200 A-JK	39.477 B-J	6230.0 ABCD	1.8656 ABCDE	47.667 ABCD
27	g27	14.373 EFGH	6.433 BCDE	44.833 B-K	45.193 A-I	4503.3 BCDE	1.6365 C-HI	38.333 B-J

28	g28	21.267 BCDEFGH	7.033 BCDE	48.833 A-K	35.997 CD-IJ	4923.3 BCDE	1.8423 A-F	47.333 A-D
29	g29	16.633 DEFGH	6.000 BCDE	52.767 A-I	36.080 C-J	4200.0 BCDE	1.6898 A-H	47.667 A-D
30	g30	31.467 ABC	9.600 AB	53.700 A-G	30.823 HIJ	6720.0 AB	1.7607 A-H	39.000 B-J
31	g31	20.967 BCDEFGH	5.933 BCDE	41.200 HIJK v	29.030 IJ	4153.3 BCDE	1.7455 A-H	39.000 B-IJ
32	g32	15.967 DEFGH	5.567 BCDE	53.467 A-H	38.610 B-J	3896.7 BCDE	1.6909 A-H	44.333 A-F
3 3	g33	16.433 DEFGH	5.433 BCDE	51.867 AB-K	40.070 B-IJ	3803.3 BCDE	1.6863 AB-H	39.667 B-J
34	g34	21.567 BCDEFGH	7.767 BCDE	52.767 A-I	35.013 D-J	5436.7 BCDE	1.6329 C-I	37.333 DE-J
35	g35	15.183 DEFGH	5.600 BCDE	47.833 A-JK	36.213 C-J	3920.0 BCDE	1.7145 ABC-H	40.000 BC-J
36	g36	16.400 DEFGH	6.233 BCDE	41.067 IJK	38.700 BC-IJ	4363.3 BCDE	1.9016 ABC	50.333 AB
37	g37	24.200 A-GH	9.200 ABC	55.000 ABC	38.247 B-IJ	6440.0 ABC	1.7498 A-FGH	41.667 A-GH
38	g38	19.367 B-H	7.267 BCDE	54.500 ABCDEF	37.920 B-IJ	5086.7 BCDE	1.6885 ABH	41.000 AB-I
39	g39	15.190 DEFGH	5.967 BCDE	42.333 EFGHIJK	40.020 BC-J	4176.7 BCDE	1.9360 A	47.333 ABCD
40	g40	28.000 A-D	9.833 AB	56.333 AB	35.503 C-J	6883.3 AB	1.6725 A-HI	37.000 DE-IJ
41	g41	18.933 B-GH	5.533 BCDE	47.467 AB-JK	28.673 IJ	3873.3 BCDE	1.5478 GHI	33.000 E-K
42	g42	12.533 FGH	3.767 E	54.167 AB-F	41.027 A-IJ	2636.7 E	1.6719 A-I	28.333 JK
43	g43	12.067 GH	3.800 E	42.933 CDE-K	32.457 EFGHIJ	2660.0 E	1.6274 CDEFGHI	38.667 B-J
44	g44	12.847 FGH	4.133 DE	51.267 A-IJK	31.740 GHIJ	2893.3 DE	1.7414 A-H	34.000 E-JK
45	g45	12.913 FGH	4.633 CDE	44.533 BC-K	35.370 C-J	3243.3 CDE	1.3896 I	32.000 GHIJK
46	g46	13.167 FGH	7.137 BCDE	41.200 HIJK	50.402 A-H	4995.7 BCDE	1.7652 A-H	52.333 A
47	g47	14.933 DEFGH	8.767 ABCD	50.800 A-JK	60.850 A	6136.7 ABCD	1.7639 A-GH	30.333 HIJK
48	g48	16.533 DEFGH	8.767 ABCD	44.800 BC-K	54.216 A-D	6136.7 ABCD	1.6097 D-HI	28.000 JK
49	g49	24.400 A-FG	12.867 A	41.400 GHIJK	52.321 A-DE	9006.7 A	1.6109 DEFGHI	35.667 D-JK
50	g50	21.467 BC-GH	10.000 AB	44.800 BCD-K	46.884 AB-I	7000.0 AB	1.8835 ABCD	36.667 D-J

Table 6. Top ten genotypes for specific traits

SL	Parameters	Genotypes
1	Plant height	g10, g6, g3, g34, g35, g40, g41, g11, g19, g8, g12,
2	No of tillers	g31, g37, g40, g26, g5, g20, g22, g1, g9, g23
3	FLA	g35, g49, g21, g36, g34, g41, g12, g50, g4, g10
4	Spike length	g12, g22, g40, g3, g9, g49, g5, g14, g11, g15
5	Spikelet/S	g28, g10, g40, g5, g50, g11, g15, g21, g24, g26
6	Grain/spikelet	g49, g19, g47, g16, g1, g21, g23, g26, g27, g27
7	Biological yield	g20, g24, g30, g40, g19, g25, g49, g37, g21, g26
8	Yield/plant	g20, g9, g50, g40, g7, g30, g3, g37, g26, g24
9	1000-grain weight	g3, g12, g40, g37, g9, g20, g38, g42, g30, g32
10	Harvest index	g47, g6, g7, g48,g49, g8, g15, g46, g16, g50
11	Yield/hectare	g20, g49, g50, g40, g7, g30, g3, g37, g26, g24
12	Spike density	g39, g25, g36, g50, g16, g10, g26, g1, g28, g6
13	Grain/spike	g46, g36, g25, g16, g26, g29, g18, g28, g39, g10

Table 7. Best 5 varieties on the basis of yield production

SL	Genotypes name	Yield/plant(grams)
1	g20	(12.900)g
2	g49	(12.867)g
3	g50	(10.000)g
4	g40	(9.833)g
5	g 7	(9.733)g

Grain per spike

Through (ANOVA) the highest value noted in genotype g49(4.6667), g19(4.0000), g47(4.0000), while the lowest value recorded in genotype g43(2.0000).

Biological yield

The analysis of variance (ANOVA) revealed significant variation. Among the genotypes the maximum value for biological yield was noted in genotypes g20(35.100gm) followed by g24(31.567gm), g30(31.467gm), while g16showed minimum Biological yield (11.133gm).

Yield per plant

The analysis of variance revealed that the genotypes g20(12.900gm), g49(12.867gm), g50(10.000gm), have highest value for yield per plant while genotype g42(3.767), has the lowest value noted.

1000-grain weight

The analysis of variance (ANOVA) exposed that the genotypes were highly significant. The genotypes g3(57.833gm), g12(57.233gm), g40(56.333gm), possess maximum thousand grain weight, while the genotype g25(39.667gm) has the lowest value of 1000 grain weight.

Harvest index

The (ANOVA) showed that the genotypes were highly significant. The genotypes that have the highest values for harvest index were g47(60.850), g6(57.107), g7(55.343), while the genotypes that showed lowest harvest index value was g14(24.840).

Yield per hectare

The ANOVA for grain yield per hectare shown highly significant ranges. It's ranged from 9030.0-2636.7. The genotype that have the highest yield per hectare were g20(9030.0), g49(9006.7), g50(7000.0), while the genotype g42 showed the lowest value for yield per hectare (2636.7).

Spike density

The analysis of variance revealed that the genotypes were highly significant. The genotypes g39(1.9360), g25(1.9350), g36(1.9016), have the maximum spike density shown while the genotype g45(1.3896), shown lowest spike density among 50 genotypes.

Grains/spike

The (ANOVA) revealed that the 50 genotypes were highly significant. The genotypes that shown the highest value For Grain Per Spike were g46 (52.333),g 36(50.333), g25(50.000), while the lowest value recorded was for genotype g3(23.667).

Molecular study

After isolation, genomic DNA was check out through gel electrophoresis if high amount of genomic DNA was present in different samples the samples were diluted using double distilled water. After dilution the samples were used for polymerase chain reaction to amplify the required genes of interest using Scot primers.

Only High-resolution DNA bands were included in the analysis using of 1kb DNA ladder. Results of amplification profiles of 4 Scot primers are presented in Fig. 3, 4 and Table 8.

Table 8. Primers used for monomorphic and polymorphic data

s.no	Primer	Sequence	MB	PB	TB	PP	band size
1	Scot 7	CATGGCTACCACCGGCCC	0	3	3	100%	300-1000
2	Scot 10	CAACAATGGCTACCAGCC	0	5	5	100%	200-700
3	Scot 13	ACGACATGGCGACCATCG	0	4	4	100%	500-1000
4	Scot 18	CATGGCTACCACCGGCC	0	1	1	100%	200-400
	Total		0	13	13		

M.B=monomorphic band, P.B=polymorphic band, T.B=total band and P.P=%of polymorphism.

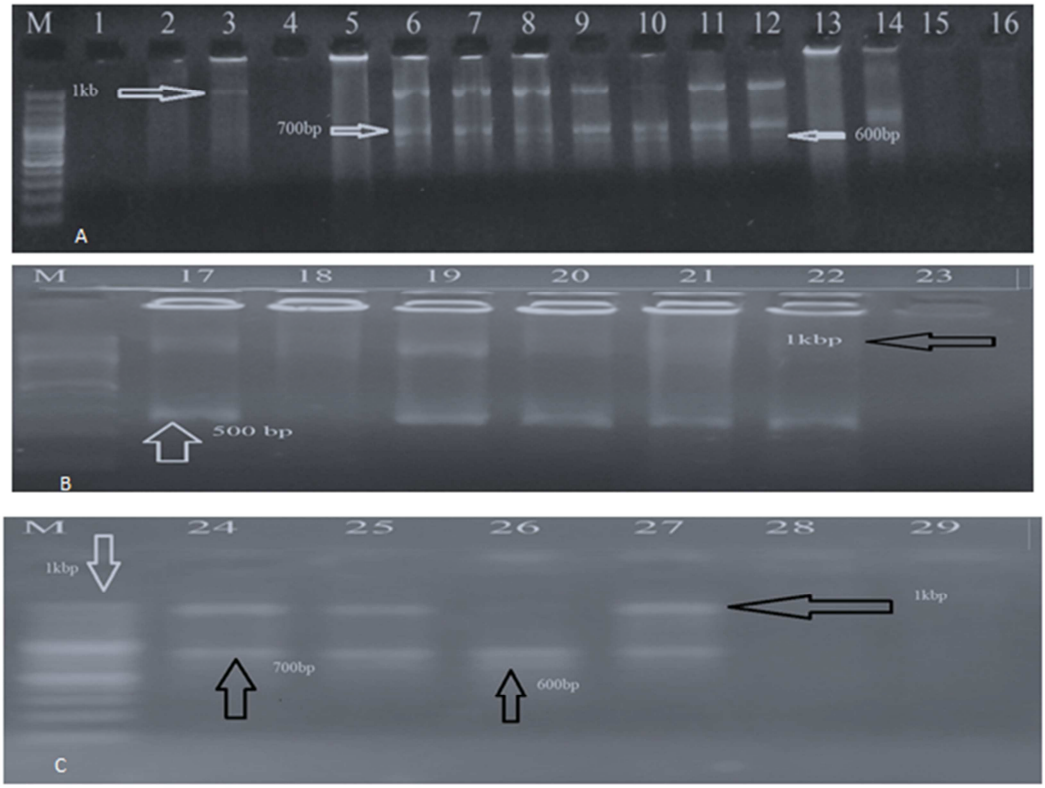

Fig. 3. (a, b and c) PCR amplification profile of 29 DNA samples of selected wheat genotypes using Scot 13 primer

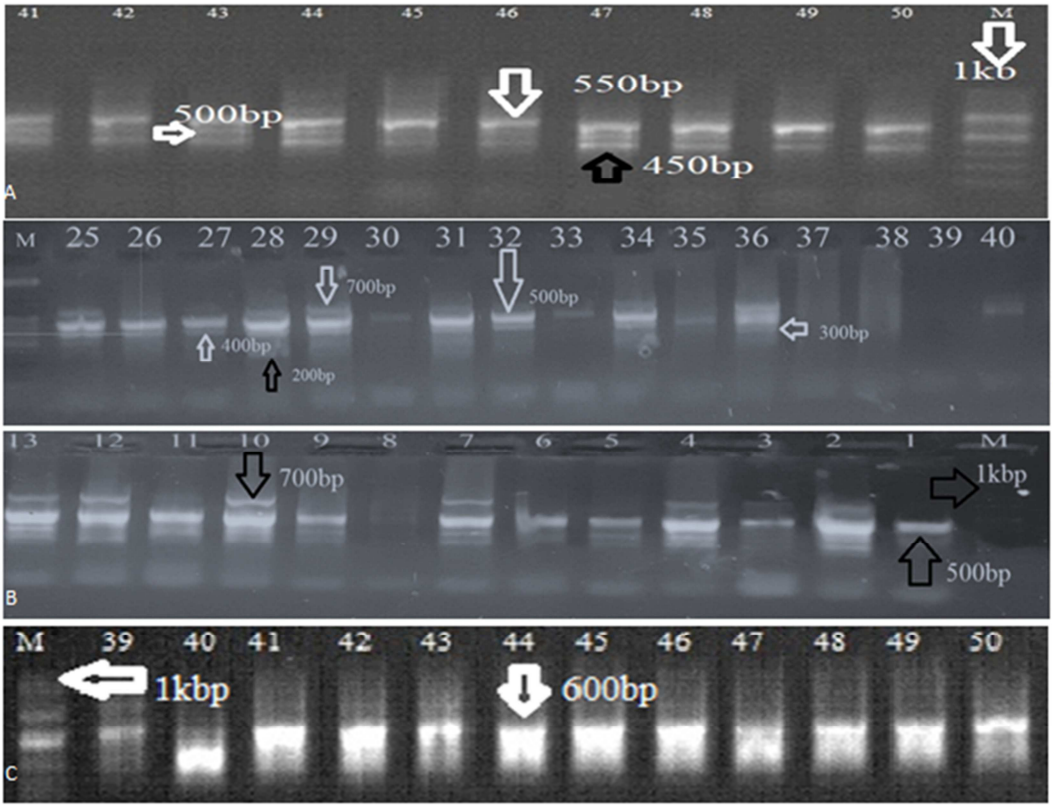

Fig. 4. (a and b) PCR amplification profile of the 40 DNA samples using Scot 7 primer. (c) PCR amplification profile of 11 DNA samples using Scot 18 primer

Polymorphism

Polymorphism is the important character in genetic analysis as all of 4 scot primers showed 100% polymorphism band size ranges from 200-1000 base pairs. Maximum bands produced by scot 13 while minimum band produced by scot 18 which was 1 in some of wheat genotypes studied.

Monomorphism

Out of 4 primers no monomorphism was shown in all 50 wheat genotypes studied.

Cluster analysis

Through DNAMAN software amplified PCR product was put to analysis. The dendogam showed homology among genotypes. The dendogram was divided into 4 groups. Group 1, 2, 3, 4 which is further divided into Sub groups.

Group 1

Comprised of 2 sub-groups, i.e., IA and IB. Sub-group IA is further divided into sub sub group of 1Aa and 1Ab. 1Aa sub group consist of genotypes g1 and g3 which is connected to each other with 100% similarity. Sub group 1Ab consist of genotype g30 and g33 which are 92% homologue with each other present independently in the group. SUB group 1B further divided into three sub groups 1Ba, 1Bb, 1Bc. 1Ba consist of genotypes g8 g14 g24 g40 that showed 100% similarity with each other 1Bb sub group consist of genotypes g15 g16, g23, g37, g38 which are 100% homologous the genotype g18 and g39 are 100% homologue and 96% similar with rest of the genotypes in the same sub group. 1Bc sub group consist of genotypes g17, g19 which are 100% similar and genotypes g20, g21, g22 showed 100% similarity with each other and 92% similar with other genotypes of the same sub group (Fig. 5).

Group 2

Group 2 comprised of 2A and 2B sub group. sub group 2A consist of genotypes g41, g43, g44, g47, g48, g49 have showed 100% similarity while 2B consist of genotypes g42, g45, g46, g50 have showed 100% similarity. Both 2A and 2B genotypes are 92% homologous (Fig. 5).

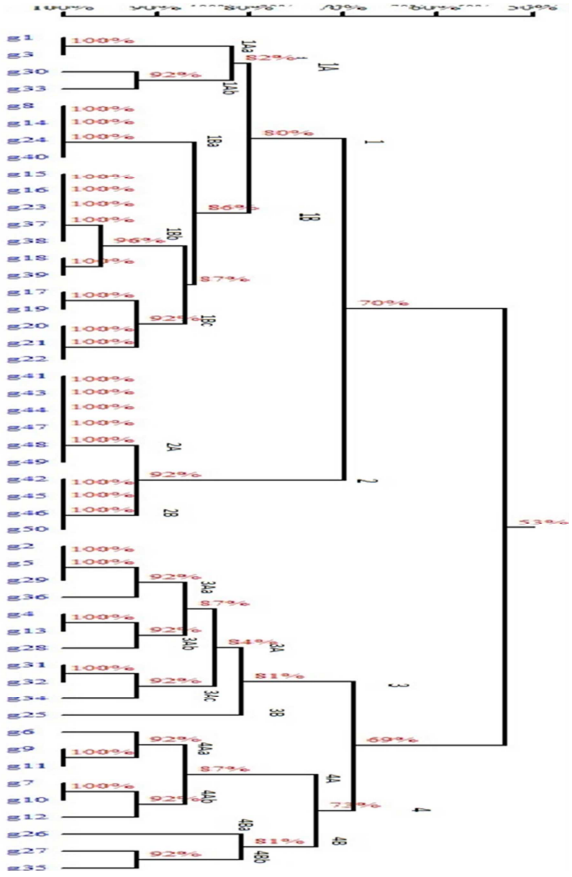

Fig. 5. Dendogram based on PCR results of wheat genotype

Group 3

Consist of two sub-groups, 3A and 3B. Sub-group 3A is further divided into 3Aa and 3Ab, 3Ac. 3Aa sub group consist of genotypes g2, g5, g29 which are 100% similar with each other while genotype g36 is present independently. Genotype g36 showed 92% similarity with other genotypes of sub group 3Aa. 3Ab sub group consist of genotypes g4, g13, which showed 100% resemblance while g28 is present independently and are interconnected with 92% similarity with rest of the 3Ab genotypes. 3Ac sub group consist of genotypes g31, g32 and showed resemblance of 100% while g34 is one present independent and showed 92% resemblance with rest of genotypes of the same group.3B sub group consist of one genotype g25 which are present independently and are showing similarity of 84% with 3A sub group (Fig. 5).

Group 4

Consist of two sub group 4A and 4B. SUB group 4A further comprised of two sub groups 4Aa and 4Ab.

4Aa sub group consist of genotypes g9, and g11 which are 100% similar while one genotype g6 is present independently and have a relation of similarity of 92% with rest of the sub group 4Aa. 4Ab consist of genotypes g7, g10 which are interconnected with inner lump of 100% similarity, while genotype g12 present independently and have a relation of homology of 92% with same sub group genotypes. 4B sub group consist of 4Ba and 4Bb. 4Ba comprised of genotype g26 which lying independently and 4Bb consist of genotypes g27 and g35 that both present independently and showed 92%similarity (Fig. 5).

Discussion

Wheat (*Triticum aestivum*) belongs to family Poaceae (Gramineae) (Brenchley *et al*., 2012). It contains protein, starch, sugar and provides food for population of the world which make it one of the most important plants in the world (Peleg *et al*., 2011; Liu *et al*., 2016). The growth of wheat and its development are influenced constantly by drought which is the basic limiting factor of yield (Yang *et al.,* 2004*)*. Drought stress is one of the performances reducing factor of wheat crop and risk for successful wheat production. Important trait related to yield is drought tolerance. The breeders required the basic changes in the set of relevant attributers to improve required trait (Yu *et al*., 2016). The main goal of breeding is to develop improved and disease resistant wheat varieties. Yield would be developed on the basis of selection of yield components which contribute to grain yield (Ashfaq *et al.,* 2003). In another study it is improved by selection for leaf area, tillers/plant, spikelet's/spike, grain weight/spike and 1000 grain weight (Munir *et al*., 2007). Morphological analysis of varieties is the most important parameter to commercialize the wheat crop (Kahrizi *et al*., 2010).

The current study was conducted in the field condition of Mansehra at Hazara University Mansehra where 50 drought tolerant genotypes were studied. The data collected for all the parameters was subjected to analysis of variance technique to estimate the differences among the genotypes (Steel *et al*., 1997). The analysis of variance and LSD test were done for morphological trait while scot primers were used for genetic analysis of wheat varieties. The ANOVA confirms significant differences between the genotypes for particular characters. Among all 50 genotypes the genotype g10(115.77A), g6(111.80), g3(107.83), Showed maximum plant height while genotype g9(72.13), showed minimum plant height. The results in terms of plant heights obtained in this research work shows similarities as described previously (Kaukab *et al*., 2014).

The maximum leaf area was documented for genotype g35(43.667), g49(42.867) and the smallest leaf area was recorded for genotype g42(26.333). Duwayri (1984) found that after the removal of flag leaves the grain yield and kernel numbers per plant were decreased. The maximum tillers per plant were noted in genotype g31(8.0000) and minimum tillers per plant were recorded in genotype g17(3.0000). The result obtained by Said *et al*., 2007 also found that tillers per plant varied from g(3.05-4.75). The genotype that have the largest spike length were g12 (11.700), g22(11.300) while genotype that have smallest spike length was g16(7.867) these results were found similar to (Rashidi, 2011). The maximum spikelet's per spike were recorded in genotype g28(19.000), g10(18.667) while minimum was in genotype g45(13.000).

The maximum grain per spikelets were in genotype g49(4.6667) g19(4.0000), g47(4.0000) while lowest grain per spikelets were noted in genotype g43(2.0000).The highest biological yield was recorded in genotype g20(35.100), g24(31.567), g30(31.467) while the lowest was recorded in g16(11.133). Ahmad *et al*., 2014 showed the same result. Highest Yield per plant was recorded in g20(12.900), g49(12.867), g50(10.000). While genotype g42(3.767) showed less yield per plant. The same result was showed by Irshad *et al.* (2012). The genotype showed maximum 1000 grain weight were g3(57.83), g12(57.233), g40(56.333), while minimum 1000grain weight was recorded in g25(39.67gm). Mohibullah *et al*., 2011 reported the same result. Highest harvest index was found in

g47(60.850), g6(57.107), g7(55.343) while lowest harvest index was recorded in genotype 14(24.840). Tripathi *et al*., 2015 showed significant difference for harvest index. Maximum yield/hectare were found in g20(9030.0), g49(9006.7), g50(7000.0) minimum yield per hectare was noted in g42(2636.7). The genotype having highest spike density were in g39(1.9360), g25(1.9350), g36(1.9016), while genotype 45(1.3896) showed lowest spike density. Maximum grain per spike were recorded in g46 (52.333), g36 (50.333), g25 (50.000), while less number of grains per spike were found in genotypes g3 (23.667) these results are in accordance with the results reported by (Knežević *et al*., 2015). Scot markers have practiced for 50 wheat genotypes. The 6 Scot markers used in the present study for 50 genotypes revealed that, 4 markers such as scot 7, scot 10, scot 13, and scot18 show high level (100%) of polymorphism. No such marker produced monomorphic bands. The DNAMAN software was used for genetic analysis of 50 wheat genotypes that produced 4 major groups 1, 2, 3, 4 which is further divided into sub groups. The overall result showed homology between the genotypes from 100% homology to 53% homology.

Conclusion

Our recent research work suggested the importance of different morphological qualities, e.g. plant height, leaf area, no of tillers per plant, spike length, spikelet's per spike, grain per spike, grain per spikelet, biological yield, yield per plant, harvest index, yield per hectare, 1000 grain weight and spike density. Those genotypes having high yield can be selected for future programme. The genotype g20 showed good performance towards biological yield, yield per plant, and yield per hectare which is recommended as a best variety towards yield and also used for breeding purpose in future.

Acknowledgments

The authors are very thankful to HEC, Hazara University and Ala-Too International University for financial support.

References

Ahmed HG, **Khan AS**, **Khan SH**, **Kashif M**. 2017a. Genome wide allelic pattern and genetic diversity of spring wheat genotypes through SSR markers. International Journal of Agriculture and Biology **19**, 1559-65.

Ahmed HG, **LI MJ**, **Khan SH**, **Kashif M**. 2019a. Early selection of bread wheat genotypes using morphological and photosynthetic attributes conferring drought tolerance. Journal of Integrative Agriculture **18**(11), 2483-91.

Ahmed HGMD, **Khan AS**, **Kashif M**, **Khan SH**. 2017b. Genetic mechanism of leaf venation and stomatal traits for breeding drought tolerant lines in wheat. Bangladesh Journal of Botany **46**(1), 35–41.

Ashfaq MU, **Khan AS**, **Ali ZU**. 2003. Association of morphological traits with grain yield in wheat (*Triticum aestivum* L.). International Journal of Agriculture and Biology **5**(3), 262-4.

Barutçular C, **Yıldırım M**, **Koc M**, **Akıncı C**, **Toptaş I**, **Albayrak O**, **Tanrıkulu A**, **El Sabagh A**. 2016. Evaluation of SPAD chlorophyll in spring wheat genotypes under different environments. Fresenius Environmental Bulletin **25**(4), 1258-66.

Brenchley R, **Spannagl M**, **Pfeifer M**, **Barker GL**, **D'Amore R**, **Allen AM**, **McKenzie N**, **Kramer M**, **Kerhornou A**, **Bolser D**, **Kay S**. 2012. Analysis of the bread wheat genome using whole-genome shotgun sequencing. Nature **491**(7426), 705-10.

Dixon J. 2009. What causes civil wars? Integrating quantitative research findings. International Studies Review **11**(4), 707-35.

Faisal S, **Mujtaba SM**, **Khan MA**, **Mahboob WA**. 2017. Morpho-physiological assessment of wheat (*Triticum aestivum* L.) genotypes for drought stress tolerance at seedling stage. Pakistan Journal of Botany **49**(2), 445-52.

Kahrizi D, **Cheghamirza K**, **Kakaei M**, **Mohammadi R**, **Ebadi A**. 2010. Heritability and genetic gain of some morphophysiological variables of durum wheat (*Triticum turgidum* var. *durum*). African Journal of Biotechnology **9**(30), 4687-91.

Kaukab S, **Saeed MS**, **Rehman A**. 2014. Genetic analysis for yield and some yield traits in spring wheat. Universal Journal of Agricultural Research **2**(7), 272-7.

Khan AD, **Khaliq I**, **Ahmad M**, **Ahmed HG**, **Khan AG**, **Farooq MS**. 2018. Comparative performance of spring wheat (*Triticum aestivum* L.) through heat stress indices. Pakistan Journal of Botany **50**(2), 481-8.

Knežević D, **Radosavac A**, **Zelenika M**. 2015. Variability of grain weight per spike in wheat grown in different ecological conditions. Acta Agriculturae Serbica **20**(39), 85-95.

Kosar F, **Akram NA**, **Ashraf M**. 2015. Exogenously-applied 5-aminolevulinic acid modulates some key physiological characteristics and antioxidative defense system in spring wheat (*Triticum aestivum* L.) seedlings under water stress. South African Journal of Botany **96**, 71-7.

Li Y, **Li H**, **Li Y**, **Zhang S**. 2017. Improving water-use efficiency by decreasing stomatal conductance and transpiration rate to maintain higher ear photosynthetic rate in drought-resistant wheat. The Crop Journal **5**(3), 231-9.

Liu EK, **Mei XR**, **Yan CR**, **Gong DZ**, **Zhang YQ**. 2016. Effects of water stress on photosynthetic characteristics, dry matter translocation and WUE in two winter wheat genotypes. Agricultural Water Management **167**, 75-85.

Liu X, **Zhu X**, **Pan Y**, **Li S**, **Liu Y**, **Ma Y**. 2016. Agricultural drought monitoring: Progress, challenges, and prospects. Journal of Geographical Sciences **26**, 750-67.

Maghsoudi K, **Emam Y**, **Ashraf M**. 2015. Influence of foliar application of silicon on chlorophyll fluorescence, photosynthetic pigments, and growth in water-stressed wheat cultivars differing in drought tolerance. Turkish Journal of Botany **39**(4), 625-34.

Mohibullah M, **Rabbani MA**, **Jahan S**. 2011. Genetic variability and correlation analysis of bread wheat (*Triticum aestivum* L.) accessions. Pakistan Journal of Botany **43**.

Mujtaba SM, **Faisal S**, **Khan MA**, **Mumtaz S**, **Khanzada B**. 2016. Physiological studies on six wheat (*Triticum aestivum* L.) genotypes for drought stress tolerance at seedling stage. Agric. Res. Technol. Open Access Journal **1**(2), 001-5.

Munir M, **Chowdhry MA**, **Malik TA**. 2007. Correlation studies among yield and its components in bread wheat under drought conditions. International Journal of Agriculture and Biology **9**(2).

Peleg Z, **Fahima T**, **Korol AB**, **Abbo S**, **Saranga Y**. 2011. Genetic analysis of wheat domestication and evolution under domestication. Journal of Experimental Botany **62**(14), 5051-61.

Rehman SU, **Bilal M**, **Rana RM**, **Tahir MN**, **Shah MK**, **Ayalew H**, **Yan G**. 2016. Cell membrane stability and chlorophyll content variation in wheat (*Triticum aestivum*) genotypes under conditions of heat and drought. Crop and Pasture Science **67**(7), 712-8.

Saeidi M, **Abdoli M**. 2015. Effect of drought stress during grain filling on yield and its components, gas exchange variables, and some physiological traits of wheat cultivars. Journal of Agricultural Science and Technology **17**(4), 885-98.

Shahinnia F, **Le Roy J**, **Laborde B**, **Sznajder B**, **Kalambettu P**, **Mahjourimajd S**, **Tilbrook J**, **Fleury D**. 2016. Genetic association of stomatal traits and yield in wheat grown in low rainfall environments. BMC Plant Biology **16**, 1-4.

Steel RG, **Torrie JH**, **Dickey DA**. 1997. Principles and procedures of statistics: a biometrical approach.

Tripathi GP, **Parde NS**, **Zate DK**, **Lal GM**. 2015. Genetic variability and heritability studies on bread wheat (*Triticum aestivum* L.). International Journal of Plant Sciences **10**(1), 57-59.

Yang J, **Zhang J**, **Liu K**, **Wang Z**, **Liu L**. 2006. Abscisic acid and ethylene interact in wheat grains in response to soil drying during grain filling. New Phytologist **171**(2), 293-303.

Yu X, **Li B**, **Wang L**, **Chen X**, **Wang W**, **Gu Y**, **Wang Z**, **Xiong F**. 2016. Effect of drought stress on the development of endosperm starch granules and the composition and physicochemical properties of starches from soft and hard wheat. Journal of the Science of Food and Agriculture **96**(8), 2746-54.

Zhu M, **Shabala S**, **Shabala L**, **Fan Y**, **Zhou MX**. 2016. Evaluating predictive values of various physiological indices for salinity stress tolerance in wheat. Journal of Agronomy and Crop Science **202**(2), 115-24.