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MOLECULAR AND MORPHOLOGICAL CHARACTERIZATION OF WHEAT GENOTYPES UNDER DROUGHT CONDITION IN NIGERIAN SUDAN SAVANNAH

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ABSTRACT

Drought stress is one of the most important abiotic constrains affecting cereal crop in the world that causes serious yield loses and threat to sustainability and food security especially in wheat thereby causing the insufficient supply of food. Therefore, understanding the genetic characterization of drought stress response is very important. This study was designed to reveal the morphological and molecular characterization of wheat genotypes using simple sequence repeat (SSR) markers and to estimate the genetic diversity and relationships among the cultivars subjected to drought conditions. A total of 15 wheat genotypes were evaluated for quantitative morphological traits such as plant height, number of seeds per spike, 1000 seed weight, spike length, flag leaf length, grain weight and proline content. Molecular characterization was done using a set of simple sequence repeat (SSR) markers. A total of 12 SSR markers were used to analyze the varieties and the genetic diversity and relationship among them. The results showed that, the drought-stressed plants had lower plant height, number of seeds per spike, 1000 seed weight, spike length, flag leaf length and grain weight than the non-stressed plants, while proline content was found to be higher in stressed plants than the non-stressed. Molecular analyses indicated significant variation among the genotypes with the mean PIC value of 0.64, mean heritability ratio of 0.67 and mean allele number of 8.9. This study also indicates the significance of SSRs as a useful tool in marker-assisted breeding about drought tolerance and for developing strategies for improving drought tolerance in cereals.

Keywords: *Wheat genotypes, Drought stress, Genetic diversity, Proline concentration, SSR markers.*

INTRODUCTION

Wheat (***Triticum aestivum*** L.) is one of the most widely grown staple diet cereal crops, contributing to the global food supply and economic security. It is one of the earliest domesticated plants and is widely accepted to originate from the levant region of the Near East and Ethiopian Highlands and provides 21% of the food calories and 20% of the protein for more than 4.5 billion people across the globe (Liu *et al.*, 2013). Cereals provide more protein to people than meat, fish, milk, and egg combined in most developing regions, except for Latin America, making them an essential source of protein for over half the world's population (FAO, 2020). Among the cereals, wheat serves significantly as a source of carbohydrate, protein, vitamins, and mineral elements. Moreover, it also is used as raw materials in industries for the preparation of alcoholic beverages, starch, and straw, or as animal feed, due to its high level of protein, carotenoid, and fiber, feeding more than 35% of the world (Colasuonno *et al.,* 2017). It is an exceptional cereal plant among recently domesticated species as it spread across all parts of the world except Antarctica after originating in the Fertile Crescent (Rasheed & Xia, 2019). It is popularly known as 'Stuff of life or king of the cereals due to its nature to occupy large acreage of land, its ranking position in the international food grain trade, and high productivity (Sharma *et al.*, 2018).

Nigeria's wheat production currently stands at about 37,200 tonnes from about 51,000 hectares, according to the Food and Agricultural Organization (FAO, 2020). Yield is an important complex trait strongly influenced by environmental conditions. Breeders are challenged to enhance the current level of wheat production due to the rapid increase in population, climate change, and environmental stresses that arose as the main threat to staple crop production. Therefore, increasing yield potential is important in solving the wheat food shortfall and meeting the rising demand for wheat grain by the ever-increasing world population (EL Sabagh *et al.*, 2021), especially in Africa. In West Africa, Nigeria is a major wheat-growing region and mostly grown under irrigation in the northern parts of the country between latitudes 100 and 140 N and altitudes 240-306m above mean sea level from November to March during the cold harmattan period, which provides the considerable low temperature for production (Olugbemi, 1990). Drought stress, insufficient water supply during the growing season, and other factors in most arid and semi-arid areas severely reduce yield. The reproductive stage of wheat is adversely affected by the depletion of the residual soil moisture, while the vegetative growth is heavily influenced by soil moisture retention owing to changes in the direction of the prevailing winds of the region; hence the stress of drought prevalence reduces wheat productivity more severely than any other environmental stress (Karim *et al*., 2000; Raza *et al*., 2019).

Drought stress (DS) is critical abiotic stress that adversely affects wheat plant growth and development. It has a negligible effect on the growth rate and

shortens the linear growth phase (LGP). LGP is directly associated with final grain mass. Climate change produces challenging conditions for producing the required quantity of crops that will meet fulfil the demand for the population (Kumar *et al*., 2020). Under extreme conditions, drought stress may severely disrupt several metabolic processes, resulting in reduced photosynthesis, delayed cell enlargement and division, and finally passed on the cells (Kramer, 1983; Chowdhury *et al*., 2021). The plant reproductive stage is heavily affected by DS due to the effect on plant metabolic processes compared with the vegetative growth phase, and the effect usually happens during flowering or anthesis, which reduces reproductive development, photosynthesis and grain yield (Karim *et al*., 2000; Araus *et al*., 2002). The study was designed to evaluate the morphological characteristics of different wheat cultivars grown during dry and rainy seasons, estimate the genetic diversity and relationships among the cultivars subjected to drought conditions using molecular markers.

MATERIAL AND METHODS

Plant Material and Study Site

The field and laboratory research were conducted at the Institute of Agricultural Research (IAR) Farm Samaru Zaria and at the African Centre of Excellence, Centre for Biotechnology Research and training Laboratory Ahmadu Bello University Zaria. The study site was located in Nigeria's Sudan Savannah ecological zone between latitudes 11º 5' to 7.9476' N and longitudes 7º 43' to 11.8020' E, with temperatures ranging from 12ºC - 32ºC Nigerian Meteorological Agency (NIMET). The area was characterized by two seasons: the rainy season with a long day length usually begins from May and ends in September, with heavy rainfalls in July and August. A short-day length that begins in October and ends in April to May characterizes the dry season. The mean annual rainfall was 756 mm, while the minimum and maximum relative humidity was 33.1 and 56.0%, respectively.

Treatments and Experimental Design

The experiments consist of fifteen different wheat varieties collected from Lake Chad Research Institute, Institute of Agricultural Research Ahmadu Bello University Zaria and Kadawa research Farm Kano. The cultivars include Lacri-4 (V1), Lacri-6 (V2), Norman (V3), Atila-50 (V4), Seri -3 (V5), Teves (V6), Rabih - 10 (V7), Hubara-1 (V8), Rabih-3 (V9), Cham-8 (V10), Cham-6 (V11), Atila1 (V12), Tesfa (V13), Bacarona-T8 (V14) and Rena-28 (V15). They are characterized by high yielding, with medium tillering ability, tolerant to stem borer, early to medium maturing habit with plant height ranging from 70-90cm. The experiment was laid out in a Randomized Complete Block Design (RCBD) and replicated three times. Thirty seeds were sown in each row, with each plot comprising of 6m row spaced at 30cm apart. The cultivars were also screened for drought tolerance by

irrigating up to the booting stage, and then the water was withdrawn for 3 weeks and resumed later. The cultivars that exhibited drought tolerance and those susceptible to drought were identified. All other agronomic practices were carried out on time to achieve a good crop stand. At maturity, the following parameters were observed and recorded, including Plant height (PH), number of seeds per spike (NS), 1000 seed weight (TSW), spike length (SL), flag leaf length (FL), grain weight (GW).

DNA Extraction

Genomic DNA was isolated from 21-day-old green leaves according to the manufacturer's protocol. Quick-DNA Plant/Seed mini prep Kit obtained from Zymo Research Corp with minor modification was purchase from Inqaba Biotech west Africa Ltd with a catalog No. D6020. The procedures are described as follows: 150g of finely cut leaves sample were added to Zymo Research (ZR) BashingBead™ lysis tube (2.0 mm) and 750 µl BashingBead™ Buffer was added to the tube and caped tightly. The sample were then secure in a bead beater fitted with a 2 ml tube holder assembled and process at maximum speed for 3 minutes using high-speed cell disrupters (FastPrep® -24). The ZR BashingBead™ lysis tube was centrifuge at 10000 x g for 1 minute using microcentrifuge. 400 µl supernatant was transferred to Zymo spin III-F filter in to collection tube and centrifuged at 8000 x g for 1 minute and the Zymo-Spin™ III-F Filter was discarded. 1200 µl of genomic lysis buffer were added to the filtrate in the collection tube from above and mixed well. 800 µl of the mixture from above step was transferred to Zymo-Spin™ IICR column in a collection tube and was centrifuged at 10000 x g for 1 minute. The flow through from the collection tube was discarded and the above step was repeated. Subsequently, 200µl of DNA pre wash buffer was added to the Zymo-Spin™ IICR column in a new collection tube and centrifuged at 10000 x g for 1 minute. Later 500 µl g-DNA wash buffer was added to the Zymo-Spin™ IICR column and centrifuged at 10000 x g for 1 minute. Zymo-Spin™ IICR column was transferred to a clean 1.5 microcentrifuge tube and 100 micro liter DNA elution buffer was added directly to the column matrix and was centrifuged at 10000 x g for 30 seconds to elude the DNA. Zymo-Spin™ III HCR filter was placed on a clean collection tube and 600 µl prep solution was added and centrifuged at 8000 x g for 3 minutes. The eluded DNA was transferred to a prepared Zymo-Spin™ III HRC spin filter in a clean 1.5 ml microcentrifuge tube and centrifuged at exactly 16000 x g for 3 minutes. The filtrate DNA is ready for PCR and other downstream application. The genomic DNA concentration and purity was measured using nano drop spectrophotometer.

PCR Cocktail

The cocktail was performed by preparing 25 µl of nuclease free water, 2* master mix with standard buffer, 0.5 µl forward primer, 0.5 µl reverse primer and 1 µl template DNA. The reaction mix was preheated at 94^{o}C for 30 seconds followed by 30 cycles of 20 seconds of denaturation at 94^{o}C, 30 seconds at 48-54^{o}C based on the primer annealing temperature and elongation at 68 ^{o}C for 1 min, after the last cycle a final step was maintained at 68 ^{o}C for 5 min to allow complete extension of all amplified fragments followed by holding at 4^{o}C until electrophoresis.

GEL Electrophoresis

2 % agarose gel was prepared by dissolving 2g of agarose in 100 ml 1X TAE buffer and heated in a microwave oven for 2 min and 1 microliter of ethidium bromide solution was added and then poured into a casting plate with a comb placed and was allowed to solidified. The sample was loaded into the wells and a larder of 100 bp was used. The gel was illuminated by UV trans- illuminator and photographed for assessing the DNA profiles.

Molecular Screening

The markers were scored for the presence (1) or absence (0) of amplified bands. Comparison of genotypes and examination of genetic relationships between genotypes were done by the help of Numerical Taxonomy and Multivariate Analysis System software (NTSYSpc, version 2.1) (Rohlf, 1998). To be able to obtain a dendrogram of wheat genotypes, the DendroUPGMA (D-UPGMA) program (http://genomes.urv.cat/UPGMA) was used. The genetic similarity index of wheat genotypes was calculated according to Jaccard (1908). SSR and ISSR marker polymorphism rates were determined using Polymorphism Information Content (PIC) values, which were calculated based on the following formula: $PIC = 1 - \sum Pij^2$, where Pi is the frequency of the *ith* allele (Anderson *et al.*, 1993). The heterozygosity (He) was calculated according to Liu and Wu (1998).

Determination of Proline Concentration

The analysis of proline concentration (PRC) in the leaves was measured by using acid ninhydrin reagent following the method described by Bates *et al.* [1973] with some modifications. The amount of PRC in response to water stress was performed at the flowering stage after the water stress was induced using the leaf sample. Fresh plant material (0.1g) was homogenized with 10 ml of 3% aqueous sulfosalicylic acid, and the homogenate was filtered using Whatman No. 2 filter paper. 1 ml of filtrate was then used for the determination of PRC by adding 1ml of acid ninhydrin and 1 ml of glacial acetic acid in a test tube. The mixture was shaken by hand and incubate to react for 1 h in a water bath at 100°C. After that it was then transferred to ice bath allowed to be cooled at room temperature until producing reddish color. 2 ml of Toluene were added to the reaction mixture, mixed vigorously in a test tube, and stirred for 15–20 seconds until separate layers were formed. The chromophore or upper toluene layer containing the color complex due to proline ninhydrin reaction was separated from the aqueous phase to another test tube and was warmed at room temperature, and absorbance was read at 520 nm by UV spectrophotometer. PRC was determined from the standard curve constructed from the known concentration of PRO and was expressed in μmol of proline per gram fresh weight of the leaf and calculated on a fresh weight basis as indicated in the following equation

$$\text{Proline concentration} = \frac{(\mu g\ \text{proline}^{-ml}) \times (\text{ml toluene}/115.5 \mu g^{-\mu mol})}{(\text{g sample}/5)}$$

Data Collection and Statistical Analysis

The recorded data during the experiments includes, Plant height (cm), flag leaf length (cm), spike length (cm), number of seed per spike, grain weight (g), thousand

seed weight (g), and proline content. The data collected on the quantitative traits was analyzed statistically using the analysis of variance (ANOVA) and coefficient of variation following the procedure of Panse and Sukhatme [1962]. The significance among treatment means were compared by employing Duncun's Multiple Range Test (DMRT) at $p \leq 5\%$ level of probability.

RESULTS AND DISCUSSION

Drought stress (DS) is a major contributing factor among other environmental stresses that causes significant yield losses by decreasing crop growth and productivity (Pour-Aboughadareh *et al.,* 2019) and has adverse effects on physiological and agronomic characters in wheat (Qaseem *et al.,* 2019). The mean performance of different wheat genotypes investigated for water stress tolerance on all measured variables shows a significant variation. The great variability can play a vital role in grain yield improvement of wheat in different breeding programs. The mean data response of all measured variables in Table 1 and Table 2 of wheat genotypes under irrigated and drought conditions indicated a significant decline in yield and related traits. This is in line with the work of Pour-Aboughadareh *et al.* (2020), who reported a decrease in yield and other traits due to drought stress. The response of all measured variables studied under irrigated and drought stress conditions is presented in Table 1 and Table 2.

Table 1. Mean performance of morphological traits of different wheat genotypes under irrigated conditions

Genotype	PH[a] (cm)	FL[b] (cm^2)	SL[c](cm)	NS[d]	1000SW[e] (g)	SW[f](g)	Proline content (µmol/g)
V1	77.17b	10.47c	9.52de	49.71f	36.51cd	303.00a	0.20a
V2	73.17c	11.10b	10.25d	62.00bcd	38.52b	280.12b	0.25a
V3	69.3bc	9.00f	9.32e	52.10ef	39.32a	227.90e	0.15b
V4	65.5g	7.52j	8.21g	63.81bc	40.21a	224.01e	0.18b
V5	62.2de	12.80a	11.10a	70.52b	38.72b	257.30c	0.13b
V6	77.3a	10.35c	9.70d	64.32bc	34.51c	301.12a	0.18b
V7	58.83ef	9.17f	8.32g	54.03def	41.70a	194.90f	0.17b
V8	65.83cd	10.47c	9.33e	65.51bc	34.02de	240.41cd	0.20a
V9	68.00c	8.80g	8.38fg	52.70ef	40.22a	279.90b	0.18b
V10	85.67a	8.20h	9.22e	64.70bc	34.80c	279.71b	0.24a
V11	77.33b	10.07d	11.21b	65.80bc	34.22d	238.42d	0.20a
V12	72.33c	9.50e	10.72bc	64.34bc	37.00b	257.02c	0.16b
V13	61.00def	9.50e	8.71ef	59.73cde	34.00d	270.00b	0.23a
V14	86.00a	13.03a	11.90a	80.73a	33.80d	189.91f	0.15b
V15	67.50c	11.00b	10.61c	66.52bc	36.72b	240.21cd	0.18b

Note: [a]PH = Plant height, [b]FL = Flag leaf length, [c]SL = Spike length, [d]NS = Number of seeds per spikes, [e]1000 SW = Thousand seed weight, [f]SW = Seed weight per plot

The mean variation of plant height (PH) ranges from 53.33 to 86.00 (cm) for both irrigated and drought condition. The genotype *V14* has the highest PH mean of 86.00 cm and 75.32 cm and *V7* with the lowest mean of 58.83 and 53.33 (cm) in both irrigated drought conditions respectively. The mean PH of the wheat genotypes has significant decrease of 6.8% under water stress compare with irrigated condition (Table 1 and Table 2) which could be attributed to the environmental factors (Abdulkerim *et al.*, 2015), and genetic makeup of the varieties (Shahzad *et al.*, 2007). Tefera *et al*. (2021) reported a significant reduction of 26% in PH under water stress compared with irrigated condition. Similarly, PH reduction in PH of 14% decrease due to drought was also identified by Bayoumi *et al.* (2008). Reduction in PH due drought stress was reported by Gupta *et al.* (2001). In addition, the highest mean performance in number of seeds per spikes (NS) was recorded in genotypes *V14* (80.73) and *V2* (63.50), with the lowest mean values of 49.71 in *V1* and 32.67 in *V4* for both irrigated and drought stress conditions respectively. The NS is economically important and has a direct measure on yield. this finding is in line with Kumar *et al.* (2020), who reported a significant decrease in NS under drought condition.

Table 2. Means values of morphological traits of different wheat genotypes under drought conditions

Genotype	PH[a] (cm)	FL[b] (cm^2)	SL[c](cm)	NS[d]	1000SW[e] (g)	SW[f] (g)	Proline content (µmol/g)
V1	61.71de	10.30de	8.54e	47.01e	33.51d	217.11d	0.51a
V2	70.53b	9.71ef	9.42cde	63.50a	33.00de	206.20e	0.32c
V3	65.67g	8.74g	8.05h	42.50efg	34.17cd	116.97h	0.37b
V4	58.33ef	8.72g	8.50d	32.67g	35.17bc	179.21f	0.40a
V5	56.17f	10.17ef	9.65bc	40.67efg	34.67bc	253.63a	0.36b
V6	60.83def	8.07g	8.37cd	50.67bcd	34.33cd	246.63a	0.49a
V7	53.33f	7.00h	8.58d	39.33fg	37.06a	184.73f	0.27c
V8	60.80de	9.91ef	8.37f	37.17gh	33.33de	232.50c	0.39b
V9	55.54ef	9.02g	8.40ef	40.17efg	35.17bc	239.50bc	0.45a
V10	65.52bcd	8.60g	8.78d	43.83defg	33.17e	218.07d	0.47a
V11	74.34a	11.71bc	8.88d	39.83fg	32f	231.60cd	0.43b
V12	71.33a	11.44c	8.70d	44.67cdef	35.33bc	255.20a	0.29c
V13	59.71def	9.02g	8.42ef	39.83fg	33.50de	161.80f	0.40b
V14	75.32a	12.02a	11.10a	57.17ab	31.00f	152.23fg	0.53a
V15	63.72cd	10.71d	8.32g	51.50bc	35.33bc	142.70g	0.25c

Note: [a]PH = Plant height, [b]FL = Flag leaf length, [c]SL = Spike length, [d]NS = Number of seeds per spikes, [e]1000SW = Thousand seed weight, [f]SW = Seed weight per plot

The spike length (SL), flag leaf length (FL), seed weight per plot (SW) and thousand seed weight (TSW) are directly contributed to yield components. The SL of different wheat genotype was found to be shorter in drought condition for most genotypes compared with irrigated condition (Table 2). The highest mean was recorded from the genotype V14 with 11.9 cm and 11.10 cm for both irrigated and drought conditions, while the lowest mean was recorded in V4 (8.21 cm) and *V3* (8.05cm) for both conditions respectively (Table 1). The difference in SL may be due to environment effect and genetic make-up of varieties (Shahzad *et al.*, 2007). Similarly, the highest and lowest mean value for FL was recorded in *V14* (13.03 cm^2) and *V4* (7.52 cm^2) in irrigated condition (Table 1), whereas the genotypes *V14* has the highest value of 12.02 cm^2 and *V7* with the lowest value 7.00 cm^2 (Table 2).

Accumulation of proline content (PC) under DS is one of the most common features in plants (Buttar *et al.*, 2005). Generally, genotypes are selected as drought-tolerant, having higher PC in DS than in normal conditions and the PC of all genotypes also increased under drought stress. The PC of all genotypes increases under drought condition when compared with irrigated condition. The highest mean performance was recorded in genotype *V14* with 0.53µmol/g and the lowest on *V15* 0.25µmol/g under drought condition. Whereas, the genotype *V2* has the highest mean value of 0.25µmol/g and V5 has the lowest value of 0.13 µmol/g (Table 1; Table 2). Increased PC accumulation acts as an osmotic for lessening of osmotic potential and increases water availability for many of fundamental biochemical pathways ongoing in plants and hence induces drought resistance (Ramond & Smirnoff, 2002). Liang *et al.* (2008), reported a significant increase of PC in wheat genotypes under stress condition and the accumulation of proline under DS, serve as a sensor of drought injury along with its prime role in stress tolerance mechanisms. High proline levels allow plants to attain low water potential, and thus imparts tolerance against moisture deficiency by increasing the biosynthesis of intermediate enzymes (Mwadzingeni *et al.*, 2016).

A total of 15 SSR primers were tested and twelve were polymorphic and used in this study (Table 3; Table 4; Figure 1).

Table 3. SSR primers, number of alleles, heterozygosity ratio and PIC values

Primer name	**Allele number**	**He[a]**	**PIC[b]**	**Band size (bp)**
Xwmc 695	11	0.48	0.46	201–273
Xgwm 350	10	0.61	0.56	145 - 197
Xwmc 233	10	0.68	0.64	218 - 276
Xwmc 182	10	0.58	0.56	100 - 159
Xwmc 9	10	0.58	0.56	155 -221
Xgwm 332	10	0.58	0.56	147 - 721
Xgwm 260	5	0.91	0.89	165 - 181
Xwmc 603	6	0.86	0.84	206 - 240
Xwmc 17	10	0.58	0.56	125 - 192
Xbarc 121	8	0.74	0.72	100 - 120

Xgwm 573	9	0.66	0.64	179 - 191
Xgwm 130	8	0.74	0.72	114 - 137
Total	**107**	**8.00**	**7.70**	-
Average	**8.9**	**0.67**	**0.64**	-

Note: [a]He, Heterozygosity; [c]PIC, polymorphism information contents

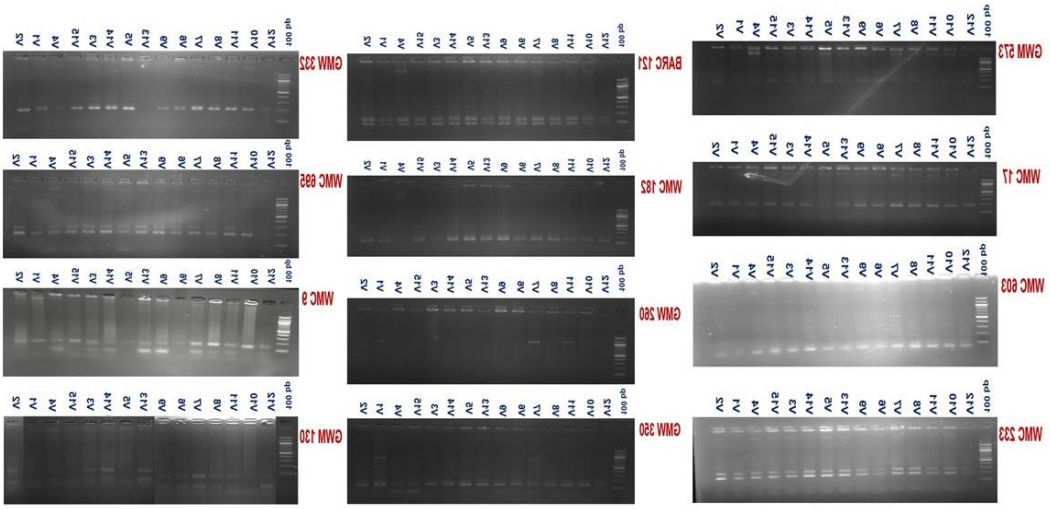

Figure 1. Gel image for molecular characterization of 15 wheat genotypes with 12 SSR primers (L:100bp)

Likewise, for the seed weight per plot (SW), the highest mean of 303.00 g and 255.20 g was observed in genotypes V1 and V12, and lowest mean values of 189.91 g and 116.97 g was recorded in genotypes V14 and V3 for both irrigated and drought conditions respectively. Under irrigation condition, wheat genotypes performed differently for 1000 seed weight (TSW). The highest TSW was recognized in genotypes V9 with 40.22 g and V14 with the lowest values of 33.80 g. whereas the genotypes V7 has the highest mean value of 37.06 g and V14 with the lowest value of 31.00 g under drought condition. Generally, under drought condition, TSW has direct effect on grain yield, leading to significant decrease in grain yield Dadbakhsh et al. (2011). The cumulative influence of environmental and genetic variables is responsible for the variations among the genotypes for all analyzed characters. Kumar et al. (2020) reported that severe water stress has a great influence in yield component. Similarly, drought stress has caused a reduction in all the yield contributing characters (Kilic & Yagbasanlar 2010). Blum and Pnuel (1990) reported that yield and yield contributing traits of wheat were rastically decreased under least annual precipitation.

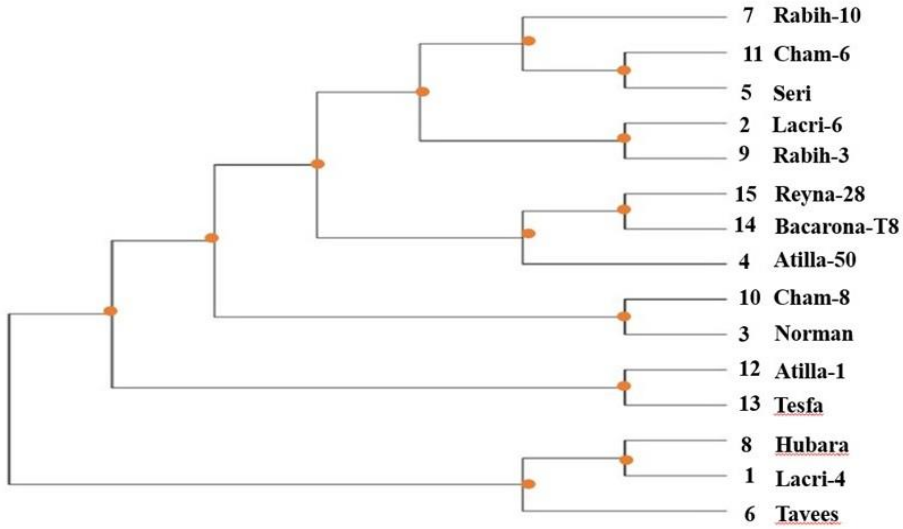

Figure 2. Dendrogram showing phylogenic relationship in a cluster for the fifteen wheat varieties

Table 4. Presence (1) versus absence (0) of PCR-amplified fragments from fifteen genotypes using twelve SSR primers

Pimer Name	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14	V15
XWMC695-3A	0	1	1	1	1	0	1	0	1	1	1	0	1	1	1
XGWM350-4A	0	1	0	1	1	0	1	1	1	1	1	0	0	1	1
XWMC233-5D	0	1	0	1	1	0	1	1	1	0	1	1	0	1	0
XWMC182-6B	1	1	0	1	1	1	0	1	1	1	0	1	0	0	1
XWMC9-7A	0	1	1	1	1	0	1	0	0	0	1	1	1	1	1
XGWM332-7A	0	1	1	0	1	1	1	0	1	0	1	1	0	1	1
XGWM260-7A	0	0	1	0	1	0	0	0	1	0	1	0	0	1	0
XWMC603-7A	1	1	0	1	1	0	0	0	0	1	1	0	0	0	0
XWMC17-7A	1	1	0	0	1	1	1	1	0	1	0	1	1	0	1
XBARC121-7A	0	0	1	0	1	0	1	0	1	1	1	0	1	1	0
XGWM573-7B	1	1	0	1	1	0	1	0	1	0	1	0	1	0	1
XGWM130-7D	0	0	1	0	1	1	1	1	0	1	1	0	0	0	1

The Polymorphic information content (PIC) was estimated to determine the genetic diversity and the level of gene variation in plants (Ateş Sönmezoğlu & Terzi, 2018; Al-Tamimi & Al-Janabi, 2019). The locus of gene is considered high diversity if the PIC value is > 0.5, while if the value is < 0.25, it is considered to be low diversity (Nagy *et al*., 2012; Ramadugu *et al*., 2015). In the present study the mean PIC values for the SSR markers ranged from 0.46 to 0.89 with the mean recorded as 0.64. The highest PIC value was observed in *Xgwm260* marker with 0.89 and the lowest was determined in *Xwmc695* with value of 0.46**.** Similarly, there was no much significant

different between the heterozygosity ratio (He) and the PIC values. The highest He was reported to be 0.91 in *Xgwm260* primer and the lowest in *Xwmc695* primer with the value of 0.48 (Table 3). The results indicates that out of the twelve SSR marker used in this study nine of them were found to be highly informative due to significant genetic variation, therefore they can be utilized to assist selection of drought stress tolerance in breeding programs. Previous studies suggest that genetic diversity in wheat genotypes should be reflected by PIC values as well as number of alleles per locus due to significant variation (Mkhabela *et al.*, 2020). These results are in accordance with Ateş Sönmezoğlu and Terzi (2018) who characterized different wheat varieties using SSR, single nucleotide polymorphisms (SNP) and randomly amplified fragment polymorphisms (RAPD) markers to screen for drought tolerance genotypes. They reported the reliability and significance of SSR markers in genetic characterization of drought tolerance in bread wheat. Other studies were conducted using 27 bread wheat genotypes to evaluate their response to drought stress through six SSR and eight ISSR markers which they reported significant genetic diversity among the genotypes which could be utilized for marker assisted selection and other breeding programs (Ates-Sonmezoglu *et al*., 2022). Yadav *et al.*, (2018) reported significant variation using 15 ISSR markers for six drought tolerance and six drought sensitive wheat varieties in which 14 markers gave reproductive bands.

Table 5. Genetic similarity values among 15 wheat genotypes

Genotypes	V1	V2	V3	V4	V5	V6	V7	V8	V9	V10	V11	V12	V13	V14
V2	0.71													
V3	0.57	0.72												
V4	0.62	0.78	0.78											
V5	0.76	0.85	0.76	0.79										
V6	**0.52**	0.61	0.58	0.69	0.67									
V7	0.71	0.76	0.69	0.72	0.71	0.67								
V8	0.56	0.66	0.59	0.65	0.72	0.56	0.79	.						
V9	0.68	0.85	0.71	0.81	0.69	0.66	0.84	0.64						
V10	0.62	0.77	0.69	0.75	0.86	0.67	0.81	0.62	0.81					
V11	0.82	0.89	0.68	0.77	**0.92**	0.64	0.89	0.65	0.86	0.79				
V12	0.54	0.58	0.60	0.62	0.71	0.56	0.69	0.61	0.76	0.68	0.68			
V13	0.55	0.57	0.59	0.62	0.72	0.57	0.70	0.61	0.76	0.68	0.68	0.57		
V14	0.62	0.73	0.63	0.69	0.80	0.76	0.81	0.65	0.85	0.76	0.72	0.62	0.67	
V15	0.68	0.76	0.63	0.70	0.85	0.77	0.86	0.66	0.86	0.76	0.73	0.65	0.69	0.77

A dendrogram was constructed among the genotypes based on the genetic similarity coefficient value using the SSR information (Figure 2). The varieties V5 and V11 gave the highest genetic similarity value (0.92). The closest genotypes were found in V5 and V11 with genetic similarity of 0. 92 (Table 5). In addition, 12 out of the 15 bands of V5 cultivar had similar amplified bands with V11 (Table 4). This implies that cluster analysis can be a useful tool for identifying drought tolerance genotypes. However, V1 and V6 varieties had the least genetic similarity value of 0.52 (Table 5) indicating that V6 is the most divergent genotype among the genotypes as it is located in separate cluster. This work is similar to the findings of (Mansour et al.,

2020) and Tahir (2008). Similarly, Tungalag *et al.* (2018), reported that ISSRs markers could be used to determine genetic relationships by using 17 ISSR markers to define variants in six Mongolian local wheat varieties.

CONCLUSION

The current study was designed to evaluate the morphological characteristics of different wheat cultivars grown during dry and rainy seasons and estimate the genetic diversity and relationships among the cultivars subjected to drought conditions using molecular markers. Identifying variations between phenotype and genotype of targeted germplasm will effectively use genetic resources. The results reveal a significant variation among the genotype with PIC values ranged from 0.46 to 0.89 with the mean value recorded as 0.64, which allowed the identification and selection of drought related genotypes. Those genotypes will be very useful genetic resources for various breeding programs especially for characterizing genotypes for drought tolerance for molecular breeding.

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