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Assessment of the Performance of Some Peanut Genotypes under Drought Conditions

Samah A. Sabry[1], I. M. Elareny[2] and Eman T. Abdou[2]

[1]Department of Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt
[2]Field Crops Research Institute, Agricultural Research Center, Giza, Egypt

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ABSTRACT

This study was conducted during the two successive seasons 2019 and 2020 to evaluate the performance of peanut genotypes under drought stress using eleven agronomic traits and six SSR (Simple Sequence Repeat) markers. The finding of the analysis of variance showed that all the phenotypic traits measured among the varieties have significant variations. Genotypic and Phenotypic coefficient of variation (GCV and PCV) were higher under stress conditions than controlled conditions excepting pods number per plant and pods-weight per plan. Heritability reached more than 94% for most studied traits with exception to the branches number per plant and pods-yield per feddan (8.5% and 70.7%, respectively) under stress conditions. Genetic advance as percent of the mean (GAM) reached more than 93.5 % for all traits under control and stress conditions excepting a number of branches per plant was 41.6% under stress condition. Four SSR markers produced 10 alleles with average of 2.5 per locus and exhibited a reasonable percentage of polymorphism (0.50). Values of PIC (polymorphic information content) varied from 0.25 to 0.78 with average of 0.56. Heterozygosity values ranged from 0.27 to 0.77 with average of 0.50. However, this result showed that SSR markers had no specific genes linked to drought tolerance. But it might indicate that these types of SSR markers are very efficient and useful to investigate a genome of peanut in further research.

Keywords: peanut varieties, drought stress, SSR markers, agronomic traits.

1. Introduction

Cultivated groundnut or peanut (Arachis hypogea L) 2n=4x=40 is an allotetraploid (AABB genomes) with sets of chromosomes four times of a haploid resulting from naturally hybridization of diploid wild species, A. duranesis (AA) and A. ipaensis (BB) (Liang *et al.,* 2017). Globally, it ranks 6th among oil seed crops such as soybean, rape seed and sunflower (Nigam, 2014 and Upadhyaya *et al*., 2005).

In many rural economies, Peanut provides nutritional security by supplements maize with proteins, vitamins, micronutrients and oil. Furthermore, the peanut crop is employed as a source of nitrogen (100-152 kg/ha N) for the soil through its ability to fix atmospheric nitrogen (Nigam, 2014).

Peanut, on the other hand, is frequently produced on sandy soils with low water-holding capacity and in locations with fluctuating rainfall. As a result, without irrigation peanut may be often subjected to drought stress. Drought is a climatological phenomenon characterized by a prolonged lack of rainfall causing soil moisture loss plant water shortage (Kramer 1980). It frequently has a negative impact on the crop by substantially reducing seed yield and plant mass production (Jalilvandy & Mehdi, 2013). Peanut is a drought-tolerant species that could cope with soil moisture deficits by minimization of plant dehydration, despite the fact that water deficit can result in significant decreases in pod yield (Pereira *et al*., 2012; Songsri *et al*.,2008).

Even though Peanut crop possesses morphological, physiological, biochemical, variety, it has narrow genetic base due to polidy barrier, self-pollination, and monophyletic origin so breeding drought-tolerant cultivars is a significant goal in most of the peanut improvement programs around the world.

Corresponding Author: Samah A. Sabry, Department of Botany, Faculty of Agriculture, Suez Canal University, Ismailia, Egypt. E-mail: samahysabry12@gmail.com

The primary impediment to progress in breeding for drought resistance in peanuts is a lack of reliable and quick instruments for assessing drought-related characteristics. Growers are looking for a dependable and quick solution to drought problems.

Breeders have traditionally relied on phenotypic selection strategies to increase particular quantitative features. Because of the effect of environment on these characteristics, such procedures can be expensive, time consuming, and labor intensive. On the other hand, previous studies on peanuts showed that the genetic variability at the DNA level is very low in this crop. Many types of markers, such as B. isoenzyme, Random amplified polymorphic DNA (RAPD), Restriction fragment length polymorphic DNA (RFLP) and Amplified polymorphic fragment length (AFLP) could not detect the polymorphism among cultivated peanuts due to genetic limitations basis (Gimenes *et al*., 2002). Hopkins *et al*., (1999) first developed the Simple Sequence Repeats (SSR) technique for peanuts, and this technique was later successfully used to detect polymorphism in cultivated peanuts (Samizadeh *et al*., 2003).

These markers are small arrays of tandemly arranged bases (one to six) distributed throughout genomes and are inherently abundant, informative, and codominant. Lately, SSR markers have been renowned as useful tools in plant breeding programs such as genetic diversity analysis, genome mapping, QTL analysis, and are applicable for marker-assisted selection (Varshney *et al*., 2005a, 2005b, and 2009).

The objectives of this work were to:

1- Examine four peanut varieties response to drought stress,
2- Identify relevant drought tolerance related traits,
3- Evaluate Simple Sequence Repeat (SSR) markers across the peanut varieties

2. Materials and Methods

2.1. Field experiment

Field experiments were conducted at the Experimental Farm, Ismailia Research Station; Oil Crops Section- Agricultural Research Center (A.R.C) during 2019 and 2020 sessions. The experimental materials comprised four peanut varieties. The name and pedigree of the studied varieties are listed in Table (1).

A split plot design (two-way ANOVAs) was utilized, with water regime positioned in the main plots and genotypes in the subplots, using a randomized complete block design with three replications. The normal recommended agricultural practices of peanut production were applied at the proper time. Sprinklers to provide three water regimes during plant growth supplied irrigation water. The three water regimes were well-watered (100% from ETo), intermediate (75 % from ETo) and severe water stress (50% from ETo). The experiment was separated by 4.5 m to prevent the overlapping of sprinklers for each water regime. In the two growing seasons, the amount of water which needed for irrigation was calculated according to Penman-Monteith equation (Allen *et al.,* 1998).

The area of each plot was 2.5×3×0.6 m. Peanut seeds (2-3 seeds) were deposited in rows 2.5m length, 60cm width and 20cm apart between plants within rows. After a week had passed from planting, the plants had fully emerged then the plants were thinned to a single plant per hill. Banding on one side of the row at a depth of 5 cm, the chemical fertilizer NPK prescribed doses were sprayed at sowing. Varieties of peanuts were evaluated based on the following characteristics: Stem height (cm), number of branches per plant (cm), pods weight per plant (g), 100- pod weight (g), number of seeds per plant, seed weight per plant (g), 100 seed weight. They were all measured from a random sample of ten guarded plants from each plot during harvest. and oil percentage (%) It was determined according to A.O.A.C (1984) methodology using a 5.0 g sample from each treatment.

SPAD chlorophyll reading: The Leaf's Contents of chlorophyll was determined by measuring chlorophyll content. Chl. content was measured in three separate samples from each plant using a handheld leaf Chl. meter (SPAD-502; Spectrum Technologies, Plainfield, IL). The Chl. metre displays the amount of chlorophyll present in a leaf as a whole.

2.2. Statistical analysis

A randomized complete block design (RCBD) with three replications was used in a spilt plot design arrangement, Because of variations in drought severity, the results of each field trial were

analyzed independently. Statistical Analysis Two-way analysis of variance (ANOVA) was carried out as recommended by Steel *et al.,* (1997). After conducting a homogeneity test, we used a combined analysis of variance to the data collected throughout the two seasons. A computer program Genstat 8 Rel.PL16 was used for data analysis, and the results confirmed the relative importance of the various features based on a set of genetic criteria (both genotypic and phenotypic).

Table 1: The name and pedigree of the peanut genotypes

Code	Genotype	Origin	Grow hobit	Days to maturity
1	Var. 198	U.S.A	Erect	110
2	Giza 6	Egypt	Erect	120
3	Var.276	China	Semi spreeding	115
4	Var.267	China	Erect	120

2.3. Molecular analysis

2.4.1. Plant material and DNA extraction

Genomic DNA isolation required growing seeds of each genotype to the four-leaf stage, DNA extraction by DNeasy plant minikit (Quigen Inc., Cat.no.69104, and USA). At 260nm, an ultraviolet (UV) spectrophotometer was used to determine the DNA concentration in the final samples. Electrophoresis in a 1% agarose gel in TAE buffer was used to examine the DNA.

2.4.2. SSR - Polymerase chain reaction (PCR) Procedure

As shown in (Table2), six SSR markers were selected based on their described by Hopkins *et al.,* (1999). 25 µL reaction mixture was used for DNA amplification in PCR tubes, including, having 1 µL template DNA, 1 µL SSR primer, 15 µL of dd H2O and 7 µL PCR mix. The following protocols were used in a PTC- 200 thermal cycler (MJ Research, Watertown, USA) for amplification: Beginning with DNA denaturation at 94°C for 2 minutes, the temperature profile consisted of 35 cycles at the following times and temperatures: 94°C for 45 seconds, 56-62°C for one minute, and 72°C for one minute and thirty seconds. Each SSR had its annealing temperature fine-tuned independently. Samples were incubated at 72°C for 10 minutes following the final cycle to achieve full extension. A one-hour, 80-volt gel electrophoresis run was carried out in the Pharmacia Submarine (20 x 20 cm). Gel documentation 2000, Bio- Rad, was used to capture images of the bands that were spotted on a UV-transilluminator.

2.4.3. SSR data analysis

Each peanut genotype and set of SSR primers was given a distinct visual score for the presence or absence of the SSR (simple sequence repeat) bands. SSR analysis scores for estimated observed number of alleles per locus utilizing data from all polymorphic primers (Na). The following formulas were used to ascertain the percentage of polymorphism and the heterozygosity He or PIC. The polymorphism percentage is calculated as follows: polymorphic bands/total bands in that assay unit x 100. The following formula, presented by Powell *et al*. (1996), was used to calculate PIC. In this formula, PIC = [1-Σfi2], where f is the average allele frequency across loci.

Table 2: Name and sequence of 6 SSR primers which were used for SSR-PCR analyses**.**

Primer name	Sequence
Ah8- SSR 1	Forward 5ATCAT TG T GCT GA GGGAAG3’ Reverse 5’CACA TT TTT CTTT TTC AC 3’
Ah9- SSR 2	Forward 5TCA ACT TTG GCT GCT TCC TT3’ Reverse 5’TCA ACC GTT TTT CAC TTC CA 3’
Ah10- SSR 3	Forward 5’ATC ACC ATC AGA AGG ATC CC 3’ Reverse 5’TTT GTA GCC TTC TGG CGA GT 3’
Ah15- SSR 4	Forward 5’TCG GAG AAC CAA GCA CAC ACA TC 3’ Reverse 5’TTG CGC TCT TTC TCA CAC TC 3’
Ah16- SSR 5	Forward 5’CAG AGT CGT GAT TTG TGC ACT G 3’ Reverse 5’ACA GAG TGG GCC GTC AAG TA 3’
Ah20- SSR 6	Forward 5’TGG AAT CTA TTG CTC ATC GGC TCT G 3’ everse 5’CTC ACC CAT CAT CAT CGT TCA CAT T 3’

3. Results

3.1. Analysis of variance

The variance analysis shortened the mean squares of varieties, treatments and their interactions for all evaluated traits in the study (Table3). Considering the main factors, statistically significant variations were seen for all traits. suggesting that rank of varieties is different from water regime to another with the exception of number of branches and pods yield. ardab fad^{-1} for Vx Wx R factor. This indicates a high degree of genetic variability in the material to be exploited in breeding program, and that also revealed the broad ranges observed for each trait.

Table 3: Mean squares of morphological, physiological and reproductive traits in peanut varieties for combined analysis.

Characters 2020	S.O.V	Rep.	Water Regime	Error	Varieties	Vars. * W.*R.	Error
	d.f	2	2	4	3	6	18
Stem height (cm).		0.14	496.29***	2.21	17.93**	119.99***	2.12
Number of branches pl^{-1}		0.11	17.18***	0.04	2.76***	0.32	0.19
No. of pods pl^{-1}		1.02	559***	0.89	676.7***	5.96***	0.42
Pods/weight pl^{-1} (g).		2.88	5754.02***	0.47	14057.81***	686.99***	1.50
100- pod weight (g)		2.25	5175.36***	2.38	10496.5***	806.17***	5.36
No. of seeds pl^{-1}		0.18	873.89***	0.21	2752.26***	45.52***	0.37
Seed weight pl^{-1} (g).		1.14	1757.86***	1.97	4409.28***	69.04***	1.13
100 seed weight		2.81	784.81***	1.75	944.81***	20.27**	4.42
Oil percent		1.40	64.81***	0.40	37.29***	10.18***	1.12
pods yield.ardab $fad^{-1\ 1}$		0.26	106.04***	0.33	47.22***	1.61	1.32
SPAD values		0.76	83.11**	1.47	123.37***	14.30***	1.45

3.2. Genotypes performance evaluation

The experimental coefficient of variation values (Cv) was below 13 %, indicating a reasonably good experimental precision (Table 4 a, b, c) under three water regimes during both growing seasons. However, in season 1, variety 267 showed the highest SPAD values under three water regimes. While the lowest SPAD values were observed on Giza6. In season 2, the highest SPAD values were observed on variety 189 under tree water regimes (Table4).

To utilize any local or introduced varieties effectively in breeding for drought tolerance, it is necessary to characterize and evaluate these varieties for desirable traits (Table 4). It is obviously that Var.276 possessed higher mean values for stem height (58.5 & 70.4 cm) at100 % ETo moisture level for both growing seasons. Even though it had a higher value for stem height (48.5, 45. 4) at 75% and 50% of ETo moisture levels, respectively, for the year 2019 only and had decreased by 2020. The height of the stems of the other varieties (Var.189, Giza6 and Var.267) is less in the first season than in the second season at three different water regimes. There was an increase in the mean values of the number of branches per plant in the second year for 100/ and 75 level. It almost was unchanged in both growing years for 50 level.

There was a significant difference among the water regimes as well as varieties of peanut for the number of seeds and seed weight per plant and 100 seed weight (Table 3). Noteworthy, the three yield traits have declined dramatically by an increased lack of moisture water regime (Table4). At 100 moisture level, the highest average for each of the number of seeds, seed weight per plant and 100 seed weight (98 seed, 160g and 176.3g, respectively) was recorded to variety 189 in the first season. The same trend was scored in the second season. Giza6 had the lowest mean for the three traits (65 seeds, 59.2 g and 134.1 g, respectively).

With respect to oil content, the performances of the varieties were much better in the second season than in the first season overall at three different water regimes that, excepting a variety267 which showed increased oil content in the first season at the first water regime.

Table 4a: Performance of peanut varieties under 100 % ET_o moisture level.

Characters	Season	Var. 189	Giza 6	Var. 276	Var. 267	C.V. %	L.S.D. 0.05%
SPAD values	2019	45.87	43.47	45.6	52.3	1.84	1.72
	2020	52.91	43.13	39.21	49.41	1.62	1.49
Stem height (cm).	2019	41.27	46.3	58.5	49.6	1.62	1.59
	2020	53.83	61.67	70.43	53.33	1.09	1.31
Number of branches pl^{-1}	2019	5.5	5.17	4.53	4.9	4.83	0.48
	2020	6.83	5.10	5.50	6.83	9.47	1.13
No. of pods pl^{-1}	2019	76.0	50.0	57.5	60.0	0.92	1.12
	2020	63.0	43.0	50.5	53.5	1.06	1.12
Pods. weight pl^{-1} (g)	2019	194.4	90.73	108.53	128.77	0.67	1.74
	2020	208.17	81.77	111.8	103.77	0.88	2.22
100- pod weight (g)	2019	286.4	206.5	212.77	254.93	0.82	3.93
	2020	342.47	229.0	238.9	252.6	0.91	4.81
No. of seeds pl^{-1}	2019	98.0	65.0	80.5	93.5	0.30	0.50
	2020	109.5	66.0	67.0	75.5	0.70	1.12
Seed weight pl^{-1} (g).	2019	160.0	59.2	102.7	148.4	0.57	1.35
	2020	111.4	53.57	60.23	71.17	1.10	1.62
100 seed weight	2019	176.3	134.13	165.13	174.33	2.02	6.56
	2020	114.63	97.17	99.17	102.77	2.01	4.15
Oil percent	2019	47.25	46.45	45.47	51.93	2.89	2.76
	2020	54.89	48.55	49.5	50.00	2.45	2.49
Pods yield. ardab fad^{-1}	2019	21.25	17.29	18.59	19.42	4.69	1.80
	2020	22.42	15.51	19.27	20.42	6.01	2.33

Table 4b: Performance of peanut varieties under 75 % ET_o moisture level.

Characters	Season	Var. 189	Giza 6	Var. 276	Var. 267	C.V.%	L.S.D. 0.05
SPAD values	2019	44.4	42.33	43.83	44.4	1.78	1.58
	2020	47.5	43.40.6	43.07	45.03	3.17	2.79
Stem height (cm).	2019	36.43	44.33	48.53	36.6	2.78	2.30
	2020	53.67	51.43	45.77	56.03	3.00	3.12
Number of branches pl^{-1}	2019	4.17	3.87	3.97	4.17	5.04	0.41
	2020	5.27	4.47	4.87	5.87	6.89	0.70
No. of pods pl^{-1}	2019	67.0	25.5	29.5	58.5	2.54	2.29
	2020	56.0	36.3	40.5	43.17	1.21	1.07
Pods. weight pl^{-1} (g)	2019	133.37	33.2	52.3	100.67	1.39	2.22
	2020	142.9	81.67	86.07	92.80	1.49	3.0
100- pod weight (g)	2019	219.03	181.5	192.9	195.0	1.27	5.00
	2020	277.03	234.4	234.93	244.6	0.72	3.55
No. of seeds pl^{-1}	2019	80.0	43.5	59.0	75.5	0.87	1.12
	2020	94.83	56.0	64.0	72.33	1.07	1.53
Seed weight pl^{-1} (g).	2019	113.7	34.9	65.7	103.17	1.45	2.30
	2020	92.0	44.5	50.6	64.57	1.88	2.36
100 seed weight	2019	166.27	102.7	144.57	145.7	3.87	10.81
	2020	111.03	87.07	89.2	96.73	2.27	4.35
Oil percent	2019	49.14	48.37	44.29	47.46	2.27	2.15
	2020	51.37	50.12	44.64	50.59	2.20	2.16
Pods yield. ardab fad^{-1}	2019	18.43	14.44	14.97	16.09	11.91	3.80
	2020	19.58	13.78	16.21	16.97	8.48	2.82

Table 4c: Performance of peanut varieties under 50 % ET_o moisture level.

Characters	Season	Var.189	Giza6	Var.276	Var.267	C.V.%	L.S.D.0.05
SPAD values	2019	41.6	37.43	42.27	44.2	1.69	1.39
	2020	43.3	40.1	38.57	41.77	3.32	2.71
Stem height (cm).	2019	32.27	38.3	45.4	34.3	2.76	2.07
	2020	46.43	44.6	46.9	50.33	3.98	3.74
Number of branches pl^{-1}	2019	3.77	3.5	3.63	3.87	6.62	0.49
	2020	4.0	3.3	3.4	3.8	9.90	0.72
No. of pods pl^{-1}	2019	50.83	21.0	22.17	41.0	2.76	1.86
	2020	52.5	30.5	36.0	37.0	2.13	1.66
Pods. weight pl^{-1} (g)	2019	88.03	28.17	32.83	65.87	2.41	2.59
	2020	133.83	58.17	64.67	74.5	1.22	2.02
100- pod weight (g)	2019	196.47	145.03	170.7	175.2	1.1	3.76
	2020	269.1	202.8	208.57	216.87	1.19	5.34
No. of seeds pl^{-1}	2019	73.5	25.5	35.0	69.5	1.1	1.12
	2020	83.33	49.5	51.0	66.0	0.77	0.96
Seed weight pl^{-1} (g).	2019	84.83	23.23	30.83	77.67	2.77	2.99
	2020	74.67	31.33	36.4	57.23	2.33	2.32
100 seed weight	2019	145.17	111.13	102.53	125.7	2.49	6.02
	2020	104.9	76.53	82.97	84.83	2.35	4.10
Oil percent	2019	46.32	43.42	41.27	42.85	2.93	2.54
	2020	47.77	44.66	45.62	46.61	1.75	1.62
Pods yield. ardab fad^{-1}	2019	13.33	10.63	11.8	12.47	12.04	2.90
	2020	15.2	11.27	13.8	13.6	5.74	1.54

3.3. Analysis of the genetic parameters

Under control conditions, each trait of the investigated traits in this study had the same value for genotypic and phenotypic coefficient of variation in both growing seasons except number of branches and pods –yield per feddan had higher values of PCV than GCV (Table 5). Under stress conditions, small differences in PCV and GCV were observed for SPAD values, Stem height, number of branches and pods, oil percent and pods –yield per feddan. Remarkably, the PCV and GCV were higher under stress conditions than non-stress conditions excepting number of pods per plant and pods-weight per plan.

Measurement of broad sense heritability in this study showed the reliability of the physiological and agronomical traits as a guide to its genetic importance. Heritability values recorded above 82% for all studied traits except number of branches per plant (64%) under control condition (Table 5). On the other hand, heritability reached more than 94% for most studied traits with exception to the number of branches per plant and pods-yield per feddan (8.5% and 70.7%, respectively) under stress conditions in the first season. Obviously, during the second season, most traits maintained their high heritability values expecting stem height and SPAD that waved their values compared to the first season (Table 5). While the number of branches per plant had the same pattern as the lowest value of heritability (8.5% and 31.1%) in first and second seasons respectively under stress condition.

Genetic advance values ranged from 126.5 for SPAD to 1153.2 for weight of per plant under control condition. The number of branches per plant, oil content and pods-yield per garden had the lowest values (15.6, 56.4 and 58.2 respectively) for genetic advance under control condition. Concerning stress condition, genetic progress was very high for eight traits and low for the other four traits in both seasons. While, stem height recorded value of genetic advance (118.7 and 44.0) in the first season and in the second season respectively.

Under control and stress conditions, genetic advance as percent of the mean (GAM) reached more than 93.5 % for all traits in both seasons excepting a number of branches per plant was 41.6% under stress condition.

The all studied traits recorded different values of gain by selection (GS), ranging from 1.2 to 115.3 (Table 5) under control condition. The low and high values were obtained from a number of branches per plant and weight of pods per plant, respectively. In the first season, a number of branches per plant had a zero value of gain by selection under stress condition. Whereas, the rest of traits had values ranging from 3.4 to 58.4 (pods –yield per feddan and weight of pods per plant, respectively).

In the second season, GS values varied from 0.3 to 71.4 (number of branches per plant and pods-weight per plant, respectively). When expressed as a percentage (GS %), pods-weight per plant had the highest value at 91.2 %. The lowest value (20.8 %) recorded to a number of branches per plant under control condition in the first season.

Weight of pods per plant had the highest value in both seasons under stress. The low values (1.2 and 7 %, respectively) were found for a number of branches per plant in the first season and for stem height in the second season under stress condition (Table 5).

Table 5: Variability parameters, heritability, genetic advance, genotypis and phenotypis coefficient of variation for different characters at two different drought regimes.

Characters	Season	Water regime	Mean	GCV%	PCV%	H^2%	GA	GAM%	GS	GS%
SPAD values	2019	Control	46.2	13.3	13.4	98.5	126.5	274.0	12.6	27.2
		50 % W.	41.4	6.8	7.0	94.2	58.1	140.5	5.6	13.6
	2020	Control	46.2	13.3	13.4	98.5	126.5	274.0	12.6	27.2
		50 % W	40.9	4.6	5.7	66.1	39.0	95.3	3.2	7.7
Stem height (cm).	2019	Control	59.8	13.4	13.5	99.3	165.5	276.6	16.5	27.6
		50 % W.	37.6	15.3	15.6	96.9	118.7	315.9	11.7	31.1
	2020	Control	59.8	13.4	13.5	99.3	165.5	276.6	16.5	27.6
		50 % W.	47.1	4.5	6.0	56.6	44.0	93.5	3.3	7.0
Number of branches pl^{-1}	2019	Control	6.0	12.6	15.8	64.0	15.6	260.1	1.2	20.8
		50 % W.	3.7	2.0	6.9	8.5	1.5	41.6	0.0	1.2
	2020	Control	6.0	12.6	15.8	64.0	15.6	260.1	1.2	20.8
		50 % W.	3.6	6.6	11.9	31.1	5.0	136.9	0.3	7.6
No. of pods pl^{-1}	2019	Control	52.5	15.8	15.8	99.5	170.4	324.5	17.0	32.4
		50 % W.	33.8	43.3	43.4	99.6	300.9	891.7	30.0	89.0
	2020	Control	52.5	15.8	15.8	99.5	170.4	324.5	17.0	32.4
		50 % W.	39.0	24.2	24.3	99.2	194.3	498.1	19.4	49.6
pods. weight pl^{-1} (g)	2019	Control	126.4	44.3	44.3	100.0	1153.2	912.6	115.3	91.2
		50 % W.	53.7	52.8	52.8	99.8	584.2	1087.3	58.4	108.6
	2020	Control	126.4	44.3	44.3	100.0	1153.2	912.6	115.3	91.2
		50 % W.	82.8	41.9	41.9	99.9	714.4	862.9	71.4	86.3

Table 5: cont.

Characters	Season	Water regime	Mean	GCV%	PCV%	H^2%	GA	GAM%	GS	GS%
100- pod weight (g)	2019	Control	265.7	19.6	19.6	99.8	1072.0	403.4	107.1	40.3
		50 % W.	171.9	12.3	12.3	99.2	434.4	252.8	43.3	25.2
	2020	Control	265.7	19.6	19.6	99.8	1072.0	403.4	107.1	40.3
		50 % W.	224.3	13.5	13.6	99.2	625.0	278.6	62.3	27.8
No. of seeds pl^{-1}	2019	Control	79.5	25.7	25.7	99.9	421.2	529.8	42.1	53.0
		50 % W.	171.9	12.3	12.3	99.2	434.4	252.8	43.3	25.2
	2020	Control	79.5	25.7	25.7	99.9	421.2	529.8	42.1	53.0
		50 % W.	62.5	25.3	25.3	99.9	325.1	520.5	32.5	52.0
Seed weight pl^{-1} (g).	2019	Control	74.1	35.0	35.0	99.9	533.6	720.2	53.3	72.0
		50 % W.	50.9	47.5	47.5	99.9	498.2	979.2	49.8	97.9
	2020	Control	74.1	35.0	35.0	99.9	533.6	720.2	53.3	72.0
		50 % W.	49.9	40.0	40.0	99.7	410.8	823.0	41.0	82.2
100 seed weight	2019	Control	103.5	7.4	7.7	93.2	158.6	153.3	15.3	14.8
		50 % W.	54.1	58.3	58.4	99.8	650.5	1201.5	65.0	120.0
	2020	Control	103.5	7.4	7.7	93.2	158.6	153.3	15.3	14.8
		50 % W.	87.3	14.0	14.2	97.3	251.3	287.8	24.8	28.4
Oil percent	2019	Control	50.7	5.4	5.9	82.9	56.4	111.2	5.1	10.1
		50 % W.	121.1	15.3	15.5	97.4	382.7	315.9	37.8	31.2
	2020	Control	50.7	5.4	5.9	82.9	56.4	111.2	5.1	10.1
		50 % W.	46.2	2.7	3.2	70.5	25.8	55.8	2.2	4.7
Pods yield. (ardab fad^{-1})	2019	Control	19.4	14.6	15.8	85.5	58.2	300.1	5.4	27.7
		50 % W.	43.5	4.5	5.4	70.7	40.7	93.7	3.4	7.9
	2020	Control	19.4	14.6	15.8	85.5	58.2	300.1	5.4	27.7
		50 % W.	13.5	11.6	13.0	80.4	32.3	239.9	2.9	21.5

SSR informative

In this study 4 varieties of peanut were analyzed using 6 SSR markers (Table 6).

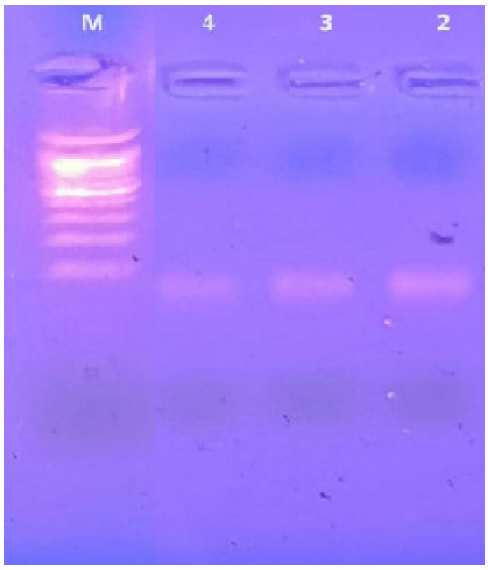
Amplification was not successful in some samples in case of AH 8 and AH15 were monomorphic amplifying a single fragment of 85 bp in each of the samples. Therefore, data of the latter two primers were omitted from the calculation.

The remaining SSR markers produced 10 alleles with average of 2.5 per locus and exhibited a reasonable percentage of polymorphism (0.50). The highest number of alleles (3) recorded to Ah 9 and Ah 1 while Ah20 and Ah 16 had 2 alleles each.

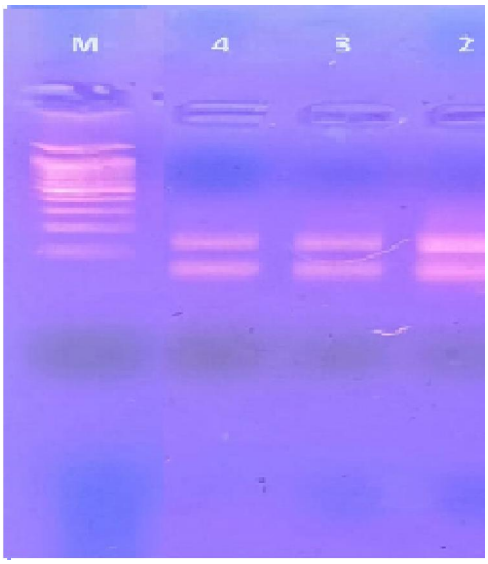
The overall size of amplified PCR products ranged from 55 – 320 bp. A microsatellite profile of each locus is shown in Fig. 1.

Heterozygosity value varied from 0.27 to 0.77 with average of 0.50. The highest (0.77) and lowest (0.27) values were detected from AH10 andAH20, respectively.

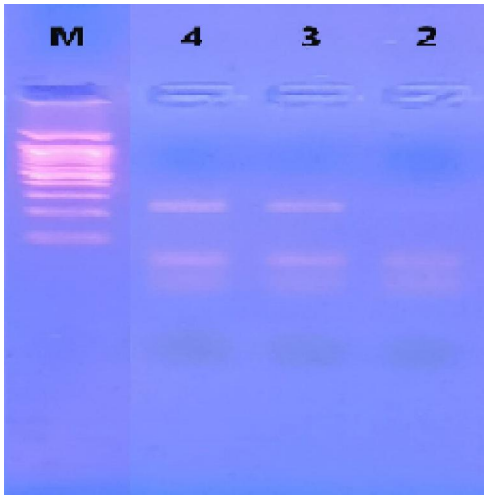
The PIC (polymorphism information content) values varied between 0.25 and 0.78 with average of 0.56. However, highest PIC value was observed with AH10 (0.78), followed by AH 9 and AH 16 (0.49) and the lowest value was recorded to AH 20 (0.25).



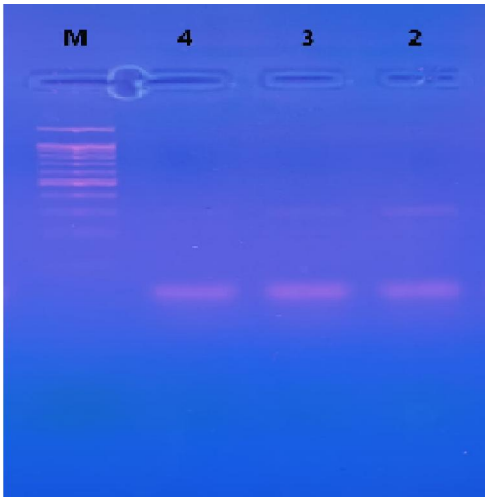
Ah8- SSR 1



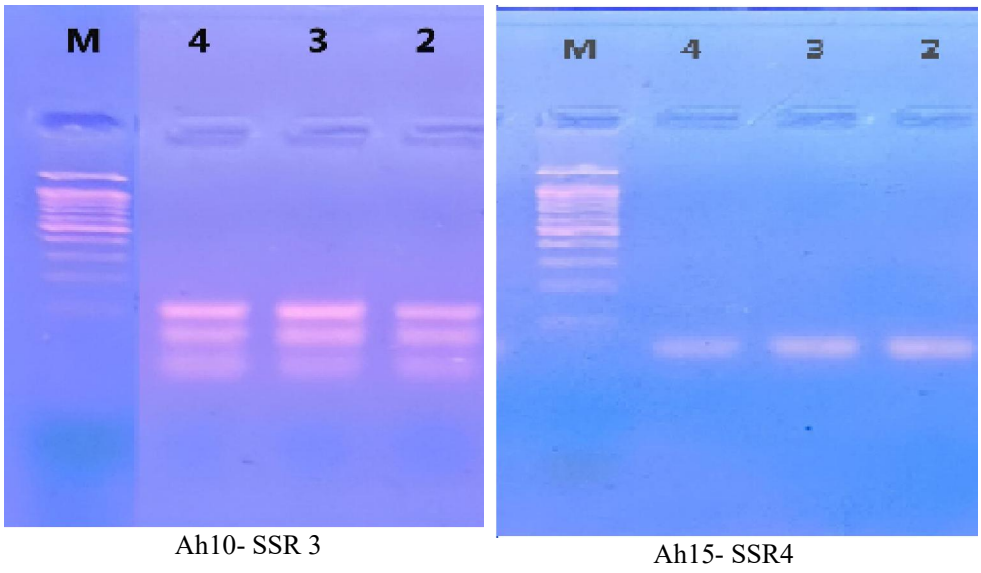
Ah20- SSR6



Ah9- SSR 2



Ah16- SSR 5



Ah10- SSR 3 Ah15- SSR4

Fig. 1: The ISSR amplification profile of primers Ah8, Ah9, Ah10, Ah15, Ah16, Ah20

Table 6: SSR Marker Informative

primers	(*na)	Alleles size rang	Heterozygosity per locus	PIC
AH 9	3	55 - 320	0.48	0.490
AH 20	2	95 - 210	0.27	0.250
AH 16	2	55 – 275[	0.48	0.490
AH 10	3	70 - 139	0.77	0.78
Total	10		2..00	2.25
Mean	2.5		0.50	0.56

4. Discussion

This study revealed wide variation for the agronomical traits useful for selection of the four tested varieties as desirable parents for peanut breeding.

The seeds number and seed weight per plant and 100 seed weight of all peanut varieties significantly decreased under the two water stress regimes.

Variations in such responses for tested peanut varieties were obviously. Vorasoot *et al.,*(2003) reported that and seed set responses of various peanut cultivars varied greatly under water stress regime, this causes a large reduction in pod yield.

The results recorded variety 267 had the highest SPAD values for three water regimes whereas variety Giza6 had the lowest SPAD values in first season. Likewise, the highest SPAD values were observed on variety 189 under three water regimes in season 2 (Table 4a, b, c). Observably, both variety 267 and variety 189 had the highest values of characters of pods and seeds under water stress regimes. This proposes that remaining photosynthetic activities under water stress might increase the pods and seed production that could represent drought tolerant in variety 267 and variety 189. According to Costa *et al.,* (2000) pointed out that the genotypes tolerated the drought stress, they had capacity to keep opening their stomata, hence ensured a high potential for CO_2 assimilation during severe water deficits. However, these results coincided with earlier findings of Falke *et al.,*(2019). This, in turn, recommends that the four cultivars are genetically very diverse, and this is a good basis for plant breeding, given that the peanut's genetic base is genetically at a lower level. As a result, these individuals could potentially be used as parents for future offspring.

High broad sense heritability contains additive and non-additive gene actions and plays a useful role in expecting a good selection as well as a large portion of variation is heritable to the offspring (Tazeen *et al.,* 2009). According to Singh (2001) who considered that the value of heritability is very high when it is greater than 80%. In this study showed some investigated traits had high heritability (> 82 %) and other traits (characters of pods and seeds) had superior heritability values (>90% -100) in

both growing seasons under both control and stress conditions (Table5) indicated they are more influenced by genetic factors rather than by environmental factors and the possibility of improvement in them. These results are in consistency with Oppong-Sekyere *et al.,* (2019) who reported that pod yield scored very high values for broad sense heritability (98.0%) in groundnuts. While moderate heritability values were recorded for stem height, SPAD and oil percent (56.6, 66.1 and 70.5 %, respectively), revealing that the influence of the environment and genotype is at the same level. The number of branches per plant is the only trait that had the lowest heritability (8.5% and 31.1%)) in first and second seasons respectively under stress conditions. So that it might not a good trait for selection. In the same way, characters of pods and seeds had high genetic advance over mean reaching more than 93.5 %.

However, in the present study showed that genetic advance (GA) was recorded very high values for majority of the traits studied. Genetic variability then occurs among the four tested peanut varieties. Worthily, it has been highlighted that without genetic advance, the heritability values would not be of practical significance in selection based on phenotypic appearance. So, genetic advance should be measured along with heritability in coherent selection breeding program. Thus, these characters showed high heritability joined with high genetic advance over mean and hence may play an essential role in drought tolerance screening to identify potential drought tolerant varieties in peanut. These characters can be used efficiently for choosing varieties with better moisture stress tolerance capacity (Pimratch *et al.,* 2010 and Pereiral *et al.,* 2015). While, low heritability with low genetic advance values was found for a number of branches per plant indicating slow progress through selection for this trait and thus, genetic improvement will be difficult. The reason for the low heritability for this component is a result of some variances constituting the environment variance (Roychowdhury and Tah, 2011).

Out of six SSR markers, only four markers created 10 alleles, ranging from 2 to 3 with an average of 2.5 per locus. The SSR markers amplified more than one locus due to the polyploid nature of the peanut crop lines, indicating locus duplication. This also proposes variability between genomes for these loci and their potential use in comparative mapping between the AA and BB genomes in peanuts. Previous studies reported amplification of more than one fragment by a pair of markers in tetraploid peanut accessions (Varshney *et al.,* 2009, Gautami *et al.,*2012 and Kamdar *et al.,*2014). This result is partly agreed with those of Nagaveni and Hasan (2019) screened 49 genotypes of peanut using 27 SSR markers that only seven markers amplified a total of 20 alleles with an average of 2.86 alleles per loci.

Concerning, the two discarded markers (AH 8 and AH15) in this study that were monomorphic amplifying a single fragment of 85 bp in each of the samples. Though, these markers succeeded in amplifying in some peanut genotypes whereas failing in other genotypes. In this study, it might be that the corresponding microsatellite sites are distantly located in the peanut DNA in such a way that no amplification occurred.

The overall size of SSR markers were used in this study that amplified PCR products ranged from 55 – 320 bp. Agreeing with Zhao *et al.,*(2012) who reported that the length of most sequences of SSR marker of peanut was ranged from 100 to 500 bp with assuming the average length of SSR containing sequences is 250 bp, these SSRs would contain 2.3 Mbp which corresponds to 0.083% of the peanut genome (2,800 Mbp). There are indicated these types of SSR markers are very efficient and useful to investigate a genome of peanut in further research.

Heterozygosity refers to the presence of different alleles at one or a lot of loci on homologous chromosomes. The locse heterozygosity (He) ranged from 0.27 in AH20 to 0.77 in AH10 with average value of 0.50. A high He average proposed that the used varieties are very heterozygous and this can be unlike self-fertilized crops like peanut. This may be owing to mutation or high natural outcrossing rate. However, it had been reportable that peanut exhibit low natural outcrossing rates starting from 0 to 8% (Mofokeng *et al.,*2021). The other reason could be these varieties were sampled from breeding population at early stages of the breeding cycle. Also, the highest value of Ho value was found at the AH20 locus could be due to high mutational rate and mutational bias at SSR loci. Loci harboring a high mutation rate are those containing a large number of simple sequence repeat units (SSR unions). As a result, any mutations in any one of the alleles may create a heterozygous condition (Bharathi, 2011). The measure of level of heterozygosity across loci can be used as an indicator of the amount of genetic variability. Zulkifli *et al.,* (2012).

The PIC values varied between 0.25 and 0.78 with average of 0.56. This PIC value is to some extent similar (0.38 to 0.75 with average of 0.53) with the previous reports of Rasam *et al.,*(2017).

Moreover, the average of PIC (0.56) among the all four peanut varieties indicated that SSR markers might have a great potential to perform the polymorphism among these varieties. However, The PIC values resulting from allelic diversity and frequency among the verities were not consistent across the SSR loci tested. This PIC value of markers could reveal maximum genetic information of the examined peanut varieties. AH10 marker had the highest value of PIC (0.78). Such a high PIC value might be due to pre-selection of the marker with the highest repeats of GC/CT. Therefore, should be taken care to verify the revealed diversity as a function of PIC value by combining additional parameters such as polymorph percentage and number of amplified alleles per locus, since quantitative estimation of marker utility and polymorphism detection with respect to the mean heterozygosity were shown (Powelle *et al.,*1996). This proposed that the loci employed were highly polymorphic and may indicate that they were highly distinctive and well suited for genetic diversity analysis (Tang *et al.,* 2007). If agro-morphological traits fail to detect variability due to similarities in growing environments, SSR markers can be a useful tool to distinguish differences between genotypes at the molecular level. Tang *et al.,* (2007) in their analysis of the genetic diversity of peanut genotypes belonging to Var.hirsuta in southern China identified all genotypes as similar based on agro-morphological characteristics. However, using SSR markers, they were capable of distinguish the variation present between peanut cultivars.

However, breeding for drought tolerance is a key focus of most breeding programs, breeding for tolerance has been difficult due to the genetic complexity of the trait, high genotyping through environmental interactions, lack of precise field-level phenotypic assessment strategies, and duration and the severity of drought in many places. Through superior biotechnological breeding, the use of SSR markers associated with water-use efficiency traits represents a powerful technique for breeding peanuts. All six SSR markers were previously used in the analysis were documented (Hopkins *et al.,* 1999, Boontang *et al.,* 2013 and Roomi *et al.,* 2014) .Yet, these indicators did not have any associations with known genes involved in optimizing water consumption. The majority of SSR markers were associated with drought tolerance features, but there were no associated genes.

5. Conclusion

Agreeing to the results of the study, most agronomic traits and observations of the SPAD chlorophyll meter can be simply and accessibly recorded under both appropriate irrigation and water scarcity conditions, facilitating the incorporation of these traits related to drought tolerance in peanut breeding and selection programs. Because of the high heritability and ease of data collection, the SPAD chlorophyll meter could be very suitable as a selection criterion for the drought tolerance of peanuts. The SPAD meter could also offer a useful tool for breeding programs to improve transpiration efficiency and possibly transpiration now. Variety 267 and variety 189 showed the potential and ability to sustain significantly high chlorophyll levels and high pod production under water stress regimes and may also show better drought tolerance. Of the six SSR markers used in this study, four detected relatively moderate levels of polymorphism. Moreover, this molecular study provided useful information for the selection of parents. The present results could benefit peanut breeders to formulate crossbreeds by selecting the studied cultivars with different genetic backgrounds and help in the development of gene mapping populations with higher marker polymorphism in future research

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