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EVALUATION OF GENETIC VARIATION AMONG SOME SORGHUM GENOTYPES USING SRAP AND RAPD MARKERS

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Abstract

The sorghum breeding programs require to select and use of diverse cultivars to create genetic diversification. Therefore, the genetic variability among six sorghum cultivars was estimated by using twenty one SRAP primers combination and fourteen RAPD primers. The total number of amplified bands was 161 and 169 of which 94 and 148 polymorphic bands which appearing 58.4% and 87.6% polymorphism for SRAP and RAPD markers, respectively. Furthermore, the values of PIC (polymorphism information content) were accounted for each primer, to estimate the discriminatory vigor of the molecular markers for analysis of linkage and polymorphism. The PIC values for SRAP markers ranged from 0.60 by Me6 + Em6 to 0.15 by Me2 + Em3 and Me2 + Em4 with an average of 0.35 and the values of PIC for RAPD markers ranged from 0.81by the OPP-08 primer to 0.41by the OPE-20 primer. Similarity coefficients among the cultivars were ranged from 78.9 % among Maka-244 and Horas cultivars to 61.3 % among the Special-90 and Maka-244. The similarity coefficient was utilized to create a UPGMA based on pooled data from SRAP and RAPD analyses to illustrate the genetic distances among the six sorghum cultivars. These analyses demonstrated the power of SRAP and RAPD markers for estimating genetic diversification among sorghum cultivars.

Key words: *Sorghum bicolor* L., SRAP marker, RAPD markers, Genetic diversity, Genetic polymorphism, Similarity coefficient, cluster analysis.

Introduction

Sorghum bicolor L. is a famous C4 grain crop with a high productivity and ranks fifth in the world grain production, it represents 55% as food for humans (grain), 33% as feed for animals (grain and biomass) and others of starch, energy, biofuels (production of ethanol alcohol), fiber (papers), dextrose syrup, fermentation (production of methane alcohol) and fertilizer (use of organic by-products) (Roy *et al*., 2018; Ruiz-Chután *et al*., 2019 and Sagar *et al*., 2019). Moreover, it is considered the staple nutrition for millions of poor human in tropical and subtropical areas around the globe because it contains of 11.3% protein, 56 to 73% starch and is rich in phosphorous, minerals, vitamin B-complex, iron and energy. In addition to it has the biological nitrification inhibition ability that can decrease the utilize of nitrogen fertilizers (El Sanousi *et al*., 2016; Roy *et al*., 2018; Raza *et al*., 2019 and Ruiz-Chután *et al*., 2019).

Sorghum is considered the most famous Egyptian summer grain yield after maize and rice for humans to fill the deficit in flour of wheat for bread production municipal and vegetative leaves as fodder for birds and animals for solving the problem of feed in summer (Khatab *et al*., 2017). According to FAOSTAT, (2017) the cultivated lands of cereal sorghum crop in Egypt was about 148,460 hectare with productively of 804,000 tons and average 5.42 ton/hectare (Khaled *et al*., 2019).

Sorghum shows a wide range of genotypic and phenotypic diversification as a result of its development across a wide range of environments (Zinzala *et al*., 2018). Wherefore, the breeders look forward to genetic diversity for the important attributes in breeding programs. This is due to the crucial role of genetic diversity in determining the selection. Thus, the evaluation of the pattern and range of genetic variation among germplasm of cultivars is a major technique for the evolution of new cultivars established on genetic similarities, so is fundamental to breeders in crop improvement programs (Khaled *et al*., 2019 and Raza *et al*., 2019). Where, genetic analysis of various cultivars can detect the range of genetic diversification and the genetic basis of complex attributes (Zinzala *et al*., 2018).

Morphological markers are highly affected by environmental conditions meanwhile molecular markers

Table 1: Names and sequences of SRAP primers used to assess the genetic diversity among the six sorghum cultivars.

Forward primer	Sequence (5'–3')	Reverse primer	Sequence (5'–3')
Me1	TGAGTCCAAACCGGATA	Em2	GACTGCGTACGAATTTGC
Me2	TGAGTCCAAACCGGAGC	Em3	GACTGCGTACGAATTGAC
Me5	TGAGTCCAAACCGGAAG	Em4	GACTGCGTACGAATTTGA
Me6	TGAGTCCAAACCGGTAG	Em5	GACTGCGTACGAATTAAC
Me10	TGAGTCCAAACCGGGAC	Em6	GACTGCGTACGAATTGCA

are not affected by such factors, therefore, the molecular markers are more efficient and decisive for evaluate the genetic diversity among cultivars and selection in breeding programs (Khaled *et al*., 2019). SRAP (Sequence related amplified polymorphism) and randomly amplified polymorphic DNA (RAPD) are techniques used to assess genetic diversity (Khaled *et al*., 2019 and Youssef *et al*., 2019). Where, Li and Quiros, (2001) introduced SRAP markers which aimed functional genes by amplifying ORFs (open reading frames) of genomes, therefore, it gives bountiful information and highly polymorphism which helps to reveal genetic diversity, thus, can be efficiently utilized for marker assisted selection (MAS) and genetic map (Li *et al*., 2014; Ge and Daizhen, 2015; Khaled *et al*., 2019 and Youssef *et al*., 2019).

SRAP is co-dominant marker technique which based on forward and reverse primers amplification. These specific primers amplified coding regions and noncoding regions of the genome by targeting CCGG exons in the forward primers and AATT introns in the reverse primers which design from 17-18 bases consist of 13 to 14 nucleotides as core sequence, Where the initial 10-11 nucleotides at 52 end are no specific, differ from primer to other and known as the "filler sequences" followed by CCGG bases in the forward primer to amplify exon regions and AATT bases in the reverse primer to amplify intron regions then the core sequence follows the 3 selective bases at the 3 end. The polymorphism among cultivars arises from difference in the length of promoter bases and introns bases (Robarts and Wolfe, 2014; Li *et al*., 2014; El Fadly *et al*., 2016 and Hassan *et al*., 2020).

Table 2: Names and sequences of RAPD primers used to assess the genetic diversity among the six sorghum cultivars.

Primer	Sequence (5'–3')
OPA-04	5'-AATCGGGCTG-3'
OPA-18	5'-AGGTGACCGT-3'
OPB-06	5'-TGCTCTGCCC-3'
OPB-12	5'- CCTTGACGCA-3'
OPC-01	5'-TTCGAGCCAG -3'
OPC-05	5'-GATGACCGCC-3'
OPD-13	5'-GGGGTGACGA-3'
OPE-20	5'-AACGGTGACC-3'
OPH-05	5'- AGTCGTCCCC-3'
OPL-04	5'-GACTGCACAC-3'
OPN-08	5'- ACCTCAGCTC-3'
OPP-13	5'- GGAGTGCCTC-3'
OPP-08	5'- ACATCGCCCA-3'
BC-526	5'-AACGGGCACC-3'

RAPD markers are characterized by highly sensitive, easy, simple, clarity, straightforward and quickness of analyze the genetic variation, similarity and evolutionary relationship between many varieties of economic crops. So, it applied to detect the polymorphisms among cultivars in different organisms. These polymorphisms are regarded as a genetic markers in different study such as fingerprinting, population differentiation and species diagnostic (Giachino, 2019; Iqbal *et al*., 2019 and Raza *et al*., 2019).

The aims of the present investigation were to assign the level of genetic variability and relationships among six sorghum cultivars by SRAP and RAPD markers to give useful information for construct phylogenetic tree among them. Also to determine the genotype-specific markers, which can provide beneficial strategies for sorghum breeding programs.

Materials and Methods

Plant Material

Seeds of six sorghum cultivars namely Maka-244, Special-85, PM, Special-90, Horas and Giza-420 were provided from Sorghum Department, Field Crops Research Institute, ARC, Giza, Egypt. Seedlings of cultivars were grown in greenhouse and the leaves of young seedlings for were used for DNA isolation.

Extraction of DNA

Genomic DNA was extracted from 0.5g of leaves of young seedlings using the CTAB method (Cetyl Trimethyl Ammonium Bromide) according to Rogers and Bendich, (1985). The DNA quantity and quality were investigated on 0.8 % agarose gel with ethidium bromide to stain of DNA.

SRAP assay

Five forward primers called "Me" which target GC rich exon regions and five reverse primers other called "Em" which target AT rich intron regions (Table 1) were used with switches and combinations to give twenty five

Table 3: Total number of scorable bands, polymorphism percentage, PIC and a band size of SRAP markers obtained by twenty one primers.

Primer	Total scorable band	Polymorphic band	Polymorphic %	PIC	Band size range
Me1+ Em2	6	4	66.7	0.47	1000-150
Me1+ Em3	**5**	2	40	0.22	750-150
Me2 + Em3	**5**	**1**	**20**	**0.15**	750-250
Me2 + Em4	6	3	50	**0.15**	1100-100
Me2 + Em5	7	5	71.4	0.35	1000-150
Me2 + Em6	8	4	50	0.32	1000-100
Me5 + Em2	9	5	55.6	0.46	1000-**120**
Me5 + Em3	8	4	50	0.38	600-130
Me5 + Em4	**11**	7	63.6	0.49	**2000**-100
Me5 + Em5	8	4	50	0.31	1700-150
Me5 + Em6	8	5	62.5	0.48	1000-130
Me6 + Em2	7	3	42.9	0.28	1500-150
Me6 + Em3	8	7	87.5	0.42	1000-150
Me6 + Em4	7	5	71.4	0.43	750-200
Me6 + Em5	8	5	62.5	0.27	1000-150
Me6 + Em6	10	**10**	**100**	**0.60**	1200-180
Me10 + Em2	**5**	3	60	0.32	600-300
Me10 + Em3	6	3	50	0.36	900-200
Me10 + Em4	**11**	6	54.5	0.23	1500-180
Me10 + Em5	8	2	25	0.19	1100-150
Me10 + Em6	10	6	60	0.37	1400-150
Total	161	94			
Average			58.4	0.35	

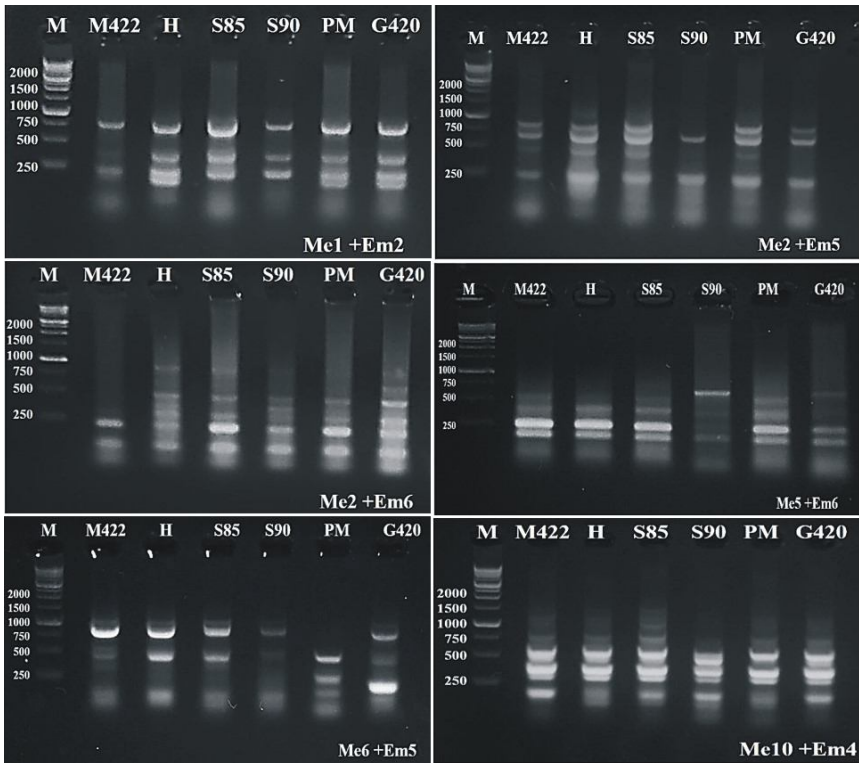

Fig. 1: SRAP banding patterns of six different sorghum genotypes ((M244) Maka-244, (H) Horas, (S85) Special-85, (S90) Special-90, PM and (G420) Giza -420), (M) 100bp and 1 kbp plus DNA ladder.

SRAP primers combinations to assess genetic diversity among six sorghum cultivars. The reaction of SRAP-PCR were carried out in 20 µl containing 10 µL Master Mix (GeneDireX), 1 µL of each primers (forward and reverse primers) (10 µM), 1 µL of DNA (10 ng) and 7 µL d.H_2O. Amplification was carried out in a thermo-cycler (Biometra, Germany) with the following program according to Li and Quiros, (2001) which consisting of 94°C for 5 min. for initial denaturation. Then the first five cycles were run at 94°C for 1 min. for denaturing, 35°C for 1 min. for annealing and 72°C for 1 min. for extension followed by other 35 cycles with raised the annealing temperature to 50°C and finally at 72°C for 5 min. The products of SRAP-PCR were separated on 1.2% agarose gel stained with ethidium bromide to stain of the amplicons.

RAPD-PCR assay

Fourteen RAPD primers were selected from Operon Technology, USA which show in table 2. The RAPD-PCR reaction were performed in 20 µl as final volume which consist of 10 µL Master Mix (GeneDireX), 1 µL of primer (10 µM), 1 µL of DNA (10 ng) and 8 µL d.H2O. The amplification was carried out in a thermo-cycler (Biometra, Germany) with the following program 94°C for 5 min. for initial denaturation followed by 35 cycles were run at 94°C for 1 min. for denaturing, 34°C for 1 min. for annealing and 72°C for 1 min. for extension and finally at 72°C for 5 min.. The products of RAPD-PCR were separated on 1.2% agarose gel stained with ethidium bromide to stain of the amplicons.

Band Scoring and Cluster Analysis

The SRAP and RAPD gel images were taken with the Gel Doc 2000 Bio-Rad system then recorded the banding patterns and determined the molecular weight of each band by 1kbp DNA ladder and then scored the data as (0)

Table 4: Sorghum cultivars and their specific SRAP markers.

Genotypes	SRAP markers		Total marker
	1	0	
Maka-244	Me5 + Em2 (120) Me5 + Em3 (130) Me5 + Em5 (600)	Me2 + Em6 (500) Me5 + Em2 (150) Me5 + Em5 (250) Me5 + Em6 (1200)	7
Horas	Me1+ Em2 (150) Me2 + Em5 (200) Me6 + Em3 (500) Me10 + Em3 (250)	Me2 + Em5 (150) Me6 + Em6 (750)	6
Special-85	Me5 + Em2 (750) Me5 + Em4 (2000) Me5 + Em6 (1000) Me10 + Em4 (1500) Me10 + Em6 (1400, 1000)	Me2 + Em4 (400, 250) Me5 + Em4 (500) Me6 + Em3 (750)	10
Special-90	Me10 + Em2 (450)	Me2 + Em4 (700) Me2 + Em5 (800) Me5 + Em3 (350) Me5 + Em5 (900,250) Me6 + Em3 (1000, 400, 250, 150) Me6 + Em4 (200) Me6 + Em5 (400) Me6 + Em6 (600) Me10 + Em2 (400) Me10 + Em3 (600) Me10 + Em4 (1200, 1000, 400) Me10 + Em6 (750)	19
PM	Me5 + Em6 (220)	Me6 + Em2 (350) Me6 + Em4 (750) Me6 + Em5 (1000,750) Me6 + Em6 (180) Me10 + Em2 (600) Me10 + Em4 (500) Me10 + Em6 (600)	9
Giza -420	Me2 + Em6 (750) Me5 + Em5 (600) Me10 + Em5 (750)	Me2 + Em5 (500) Me10 + Em4 (180)	5

absence of band and as (1) presence of the band of each cultivar. Subsequently, the coefficient of similarity between sorghum cultivars were calculated by Jaccard's coefficient of similarity (Jaccard, 1908) and construct a dendrogram (Yang and Quiros, 1993) by UPGMA method by the arithmetic average to evaluate the dendrogram using the MVSP (Multi Variate Statistical Package) version 3.22 computer program.

Results and Discussion

Sorghum bicolor L. is famous as diverse plant, therefore, its genome requires a powerful marker system for characterization (Raza *et al*., 2019). Genetic diversification is the raw material which defines the adaptive behavior of different organisms in various climates as a result of evolution. Therefore, molecular marker techniques such as SRAP marker and RAPDs marker are able to estimate the level of polymorphism, characterize the genotypes, the genetic diversification and differentiate among sorghum cultivars (Hassan *et al*., 2020). SRAP markers target functional genes, thus it have emerged as a useful molecular markers system for linkage mapping and diversification study in different species (Khaled *et al*., 2019 and Thakor *et al*., 2019). RAPD technique is a powerful molecular marker for evaluating difference within and among species, therefore, it used for taxonomic and genetic relationship among species (Zinzala *et al*., 2018; Raza *et al*., 2019 and Youssef *et al*., 2019).

In this study the genetic diversity and phylogenetic tree relationship between six sorghum cultivars were constructed by using twenty one SRAP and fourteen RAPD primers.

SRAP analysis

Twenty one diverse primer from five pairs of primers combinations were used for detecting polymorphism level in ORFs (open reading frames) to estimate the genetic diversification among six sorghum cultivars. Ninety-four Polymorphic markers were obtained from one hundred sixty-one total SRAP markers representing 58.4% polymorphism, therefore, it can be consider SRAP markers as useful markers to estimate the genetic diversity among the six sorghum cultivars. The highest number of total scorable bands was 11 which revealed with Me5 + Em4 and Me10 + Em4, while Me1+ Em3, Me2 + Em3 and Me10 + Em2 showed 5 bands as the lowest number which shown in fig. 1 and table 3. The highest number of bands is indicated that the highest number of alleles and consequently increase the diversity in genotypes and therefore the primer generates a greater number of bands the best implementation for the detection of the polymorphisms in plant genotypes.

The size of the amplified bands ranged from 120 bp

Table 5: Total number of scorable bands, polymorphism percentage, PIC and a band size of RAPD markers obtained by fourteen primers.

Primer	Total scorable band	Polymorphic band	Polymorphic %	PIC	Band size range
OPA-04	10	8	80%	0.57	1400-250
OPA-18	**18**	**16**	88.9%	0.69	1600-**150**
OPB-06	14	14	100%	0.69	1400-350
OPB-12	16	12	75%	0.63	**3000**-200
OPC-01	13	12	92.3%	0.57	2800-300
OPC-05	10	8	80%	0.60	1700-450
OPD-13	15	15	100%	0.68	1300-**150**
OPE-20	11	**7**	**63.6%**	**0.41**	1500-300
OPH-05	13	11	84.6%	0.79	2000-350
OPL-04	10	8	80%	0.56	1000-350
OPN-08	10	10	100%	0.73	**3000**-450
OPP-08	9	8	88.9%	**0.81**	1200-200
OPP-13	**8**	8	**100%**	0.76	1900-300
BC-526	12	11	91.7%	0.48	1500-200
Total	169	148			
Average			87.6%	0.64	

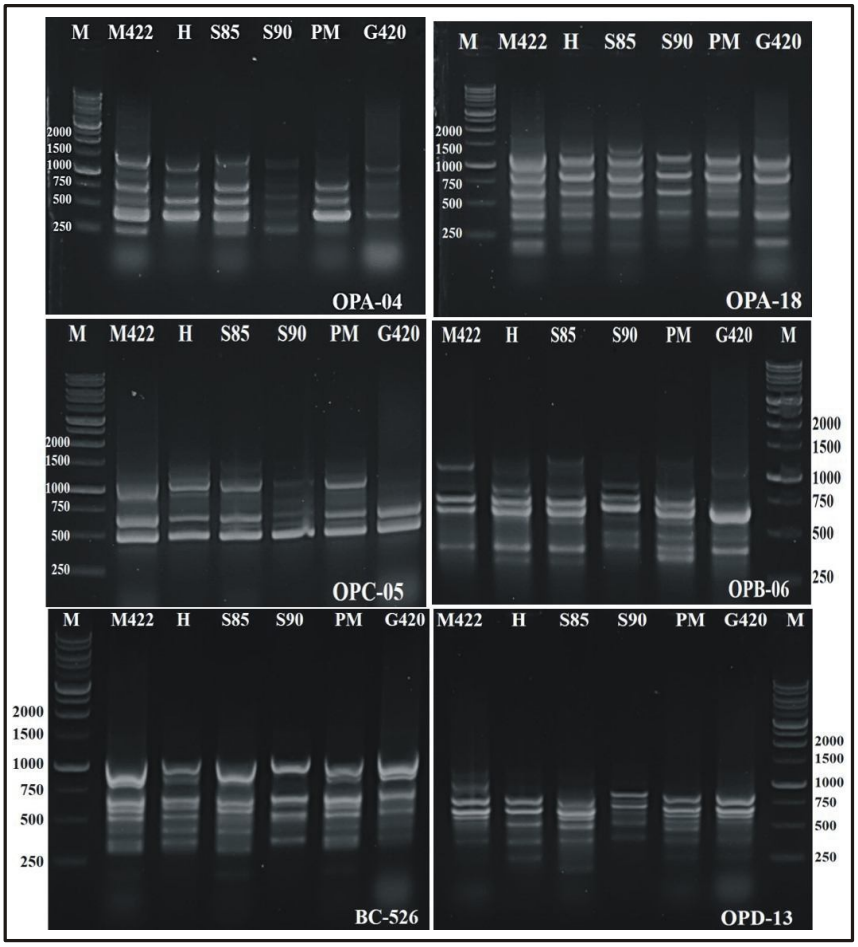

Fig. 2: RAPD banding patterns of six different sorghum genotypes ((M244) Maka-244, (H) Horas, (S85) Special-85, (S90) Special-90, PM and (G420) Giza -420), (M) 100bp and 1 kbp plus DNA ladder.

by Me5 + Em2 to 2000 bp by Me5 + Em4 and the polymorphic bands ranged from 1 with Me2 + Em3 primers which representing polymorphism 20% to 10 polymorphic bands with Me6 + Em6 primers which representing polymorphism 100%. Similarity, Khaled *et al*., (2019) demonstrated that the higher level of polymorphism percentages was 86% but the lower level of polymorphism percentages was 25% with an average 51.37% for SRAP markers among seven sorghum genotypes. In this regard, El-Hussein *et al*., (2014) showed that an average of % polymorphism was 48.5% for SRAP markers among 33 sorghum genotypes and Robarts and Wolfe, (2014) revealed that an average of % polymorphism was 68.7% among some sorghum cultivars. Khalil and El zayat, (2019) used the thirty pairs of SRAP markers to evaluate the genetic relationships between the five *Brassica* species. The percentage of polymorphism ranged from 75% to 100% with an average 93%. Also, Hassan *et al*., (2020) used 13 forward (Me1-Me13) SRAP primers and 16 reverse (Em1-Em16) primers with 208 different combinations to estimate genetic diversity among 50 wheat genotypes, where revealed that the higher level of % polymorphism was 100 while the lower level of % polymorphism was 33 with an average 55.47.

The value of polymorphism information content (PIC) for molecular markers plays an efficient role in gene mapping, genebank construction, molecular breeding and germplasm evaluation. Where, the value of the PIC is measured by the mark's ability to create polymorphism in a genotype depending on the number of alleles revealed and the frequency of distribution and therefore it is equivalent to the diversification of genes. The PIC value for molecular markers ranges from 0 to 1, where the higher the

Table 6: Sorghum cultivars and their specific RAPD markers.

Genotypes	Markers		Total marker
	1	0	
Maka-244	OPN-08 (3000) OPH-05 (2000- 1500-1000) OPA-04 (350) OPA-18 (900-750) OPB-06 (1200) OPC-05 (450) BC-526 (750)	OPA-18 (800)	11
Horas	OPN-08 (650) OPC-01 (800) OPB-12 (2000-1100) OPC-05 (1700) OPD-13 (200)	OPC-01 (1600-700-300) OPA-18 (1600) OPC-05 (650)	11
Special-85	OPH-05 (350) OPD-13 (150)	OPD-13 (750) BC-526 (500-350)	5
Special-90	OPP-08 (1200 -800) OPC-01 (550) OPA-04 (850-600) OPA-18 (1350-1000-850-400) OPB-06 (1400-850-700-425) OPE-20 (1000) OPC-05 (750) OPL-04 (1000-750) OPD-13 (900-350)	OPA-04 (500) OPA-18 (375) OPB-06 (650-400) OPE-20 (900) OPL-04 (700) OPD-13 (650-300) BC-526 (900-400)	29
PM	OPH-05 (900-300) OPP-08 (400-250) OPB-12 (1200) OPA-18 (700) OPD-13 (450)	OPA-04 (1300) OPD-13 (1300)	9
Giza -420	OPP-13 (350) OPN-08 (900) OPH-05 (1400) OPP-08 (450-200) OPC-01 (450) OPB-12 (850) BC-526 (700)	OPN-08 (800) OPB-06 (750) OPE-20 (1100) OPL-04 (900) BC-526 (650-600)	14
Total			79

number, the greater the diversity of alleles at the place of study (Chesnokov and Artemyeva, 2015; Avval, 2017 and Khaled *et al*., 2019).

The twenty one SRAP markers were utilized to evaluate their DP (differentiation power) by calculating the PIC of their loci, where the PIC values for these markers ranged from 0.60 by Me6 + Em6 to 0.15 by Me2 + Em3 and Me2 + Em4 with an average of 0.35. In this regard, El-Hussein *et al*., (2014) revealed that the PIC values ranged from 0.93 to 0.23 among 33 sorghum cultivars. Khaled *et al*., (2019) showed that the average of PIC values ranged from 0.36 by ME4FEM10R to 0.09 by ME2F-EM8R with mean of 0.17. Also, Thakor *et al*. (2019) revealed that the higher level of PIC was 0.48 by the Me5 + Em2, while the lowest level of PIC was 0.37 by the Me5 + Em1 primer with an average 0.40 for 11 markers of SRAP between 32 teak genotypes

The genotype-specific markers for six sorghum cultivars were evaluated and shown in table 4. The total genotype-specific markers were 46, where, the Special-90 cultivar scored the highest number of specific markers which was19 markers followed by Special-85 cultivar which scored ten specific markers and then nine specific markers for PM cultivar, while the lowest number of specific markers was five markers for Giza -420 cultivar.

RAPD analysis

The selected fourteen RAPD primers were used for revealing their DP (differentiation power) by calculating the PIC of their loci and the polymorphism level, showed in table 5, to estimate the genetic diversification among six sorghum cultivars. Where, these RAPD primers produced 169 total scorable bands which revealed the higher level of total bands was 18 bands which recorded by OPA-18, while the lower level of total bands was 8 bands which scored by OPP-13 and the amplicon size of these bands ranged from 150 which recorded by OPA-18 and OPD-13 to 3000 which scored by OPN-08, showed in Fig. 2, that detected a big diversity in the number of repeats among the various cultivars which led to varies of polymorphism level. Where, the OPE-20 primer showed the lowest number of polymorphic band which was 7 bands, the lowest % polymorphism which was 63.6% and the lowest PIC which was 0.41, while the OPA-18 primer revealed the highest number of polymorphic band which was 18 bands, the OPP-13 primer showed the highest % polymorphism which was 100% and the OPP-08 primer revealed the highest PIC which was 0.81. Whereas, the PIC values were accounted for each primer, to estimate the discriminatory vigor of the molecular markers for analysis of linkage

Table 7: Similarity coefficient among the six sorghum cultivars calculated according to Jaccard's coefficient revealed by pooled SRAP-RAPD analysis.

	M244	H	S85	S90	PM	G420
M244	1.000					
H	**0.789**	1.000				
S85	0.729	0.770	1.000			
S90	**0.613**	0.657	0.640	1.000		
PM	0.734	0.757	0.728	0.649	1.000	
G420	0.769	0.768	0.694	0.648	0.739	1.000

and polymorphism. Therefore, the variance in PIC values was due to the variance in diverse amplified bands created by each primer. Where, the high value of PIC indicates the further detection of the RAPD markers. So, these results revealed the power of RAPDs to differentiate among six sorghum cultivars.

Similarity, Zinzala *et al*., (2018) studied the genetic diversification among 24 sorghum genotypes using 20 RAPD markers where generated 591 total amplified bands which detected 495 polymorphic bands with 83.75% polymorphism and the value of PIC ranged from 0.69 to 0.27 with a mean of 0.52. Kanbar *et al*., (2019) analyzed the variance of genetic among 12 sorghum genotypes by 24 RAPD primers which yielded 212 total RAPD bands of which 134 were polymorphic bands with 63.2% polymorphism. Raza *et al*., (2019) estimated the genetic diversification among 30 sorghum genotypes utilizing sixteen RAPD markers which produced 132 polymorphic bands out of 148 total bands with 89.19% polymorphism and PIC values were ranged from 0.2035 to 0.3438 with an average of 0.2792. Ruiz-Chután *et al*., (2019) assessed of genetic variation in 47 sorghum bicolor genotypes by 15 RAPD markers which identified 126 total bands, 111 of them as polymorphic bands with 89% polymorphism, where % polymorphism ranged from 66.6% was scored by OPB 14 primer to 100% was scored by OPB 02, OPB 06, OPB 15 and OPB 16.

Khatab *et al*., (2017) estimated the genetic variation among 11 sorghum cultivars using six of RAPD primer and six of SRAP primer which produced fifty total bands by SRAP and thirty two total bands by RAPD which detected 27 polymorphic bands by SRAP and 29 polymorphic bands by RAPD resulting in % polymorphism ranged from 83.3% which detected by OPA12 primer to 60.60% which detected by OPA 11 for RAPD markers and 80.0% polymorphism generated by me2 + em3 primer to 20.60% generated by me1 + em3 primer for SRAP markers. Youssef *et al*., (2019) assessed the variation of genetic among five Egyptian clover cultivars using sixteen of RAPD primers and six of SRAP primers which generated 34 and 12 total bands with 24.8 and 60% polymorphism percentage of RAPD and SRAP markers, respectively.

The genotype-specific markers for six sorghum cultivars were evaluated and shown in table 6. The total genotype-specific markers were 79, where, the Special-90 cultivar scored the highest number of specific markers which was 29 markers followed by Giza -420 cultivar which scored 14 specific markers and then 11 specific markers for Maka-244 and Horas cultivars and 9 specific markers for PM, while the lowest number of specific markers was five markers for Special-85 cultivar.

These RAPD results matched with results of Zinzala *et al*., (2018); Raza *et al*., (2019) and Ruiz-Chután *et al*., (2019) and these SRAP results matched with Khatab *et al*., (2017) and Khaled *et al*., (2019) which indicated that the potential of RAPD and SRAPD markers for evaluating genetic diversification among sorghum cultivars and these information useful for stored in the Gene-Bank for future sorghum improvement programs.

The Genetic-relationships and phylogenetic tree among six sorghum cultivars

Jaccard's coefficient similarity matrix were accounted according to Jaccard, (1908) to assess the genetic diversification and relationship among six sorghum cultivars. The pooled results of SRAP-RAPD analyses revealed the highest similarity which was 78.9% among Maka-244 and Horas cultivars, while the Special-90 and Maka-244 cultivars revealed the lowest similarity 61.3%, shown in table 7. These genetic similarity among sorghum cultivars could usefulness in identification of parents for carry out heterotic crosses and achieve a maximum variation in hybridization programs resulting in developing improved sorghum cultivars (Kumar *et al*., 2017; El-Esawi *et al*., 2018 and Zinzala *et al*., 2018).

The phylogenetic tree based on the similarity coefficient detected by pooled SRAP-RAPD analysis

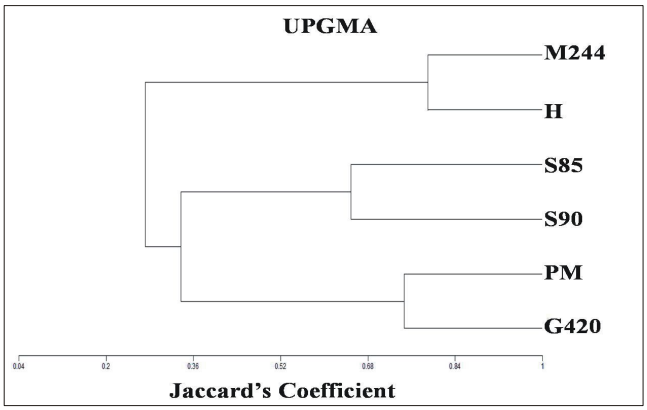

Fig. 3: Phylogenetic tree of six sorghum cultivars based on pooled SRAP-RAPD markers.

was constructed using UPGMA based cluster analysis which shown in fig. 3. The six sorghum cultivars were separated into two main clusters. The first major cluster contained Maka-244 and Horas cultivars which revealed 78.9 % similarity. While, the second cluster contained two main subclusters, the first one containing Special-90 and Special-85 which revealed 64% similarity and the second subcluster containing PM and Giza-420 which revealed 73.9% similarity

Conclusion

The results of this study confirmed that SRAP and RAPD markers could be successfully applied to estimate genetic diversification and the phylogenetic relationships among six sorghum cultivars which may help future sorghum improvement programs. Also, the genotype-specific molecular markers may be used for supporting with the new information for these programs. The variety in PIC values is due to the variation in cultivars which help the breeders to select the most diversified cultivars to rise their effectiveness for breeding of sorghum, which will be very beneficial to widen the genetic basis of the presently cultivated sorghum germplasm.

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