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Molecular markers to assess genetic variations and yield for developing climate-ready sesame cultivars

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Crop productivity is declining due to climate change, with sesame being particularly vulnerable due to its narrow genetic base. To address this, 28 advanced sesame lines were evaluated across 23 environments in Egypt over six growing seasons (2018–2023). The study aimed to identify stable and high-yielding genotypes for developing climate-ready cultivars. Additive main effects and multiplicative interaction analyses were employed to assess genotype stability and performance. Molecular markers (RAPD and ISSR) were used to characterize genetic variation and identify markers linked to yield and stability. The experiments were performed in a randomized complete block design (RCBD) with three replicates. The results showed that the genotypes had significant differences ($p < 0.05$) for seed yield. AMMI analysis identified C5.8, C3.8, C6.11, C6.2, and C9.6 as stable and high-yielding genotypes. RAPD (random amplified polymorphic DNA) and ISSR (inter-simple sequence repeat) markers revealed high levels of genetic variation; eight RAPD and ten ISSR primers amplified 78 and 84 bands with a mean polymorphism of 77.5% and 60.47%, respectively. In addition to seven markers associated with yield and stability. These findings highlight the potential of these advanced lines for developing climate-ready cultivars.

Keywords: Sesame, lines, AMMI analysis, yield stability, RAPD, ISSR, Climate-ready cultivars.

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INTRODUCTION

Climate change causes the intensity and frequency of extreme weather events like droughts, temperature rises, and floods. As a result, global agricultural productivity decreases, and the extinction rate of plant species is increasing, which could lead to environmental collapse (Teixeira & Huber, 2021; Okolie et al., 2022; Salgotra & Chauhan, 2023). Therefore, there was a need to increase genetic diversity to obtain climate-ready crops to overcome the potential effects of climate change (Hernandez-Ochoa et al., 2019; Kholova et al., 2021).

Sesame (*Sesamum indicum* L.) is grown annually for its edible seeds, oil, and taste qualities (Baraki et al., 2020). Sesame offers high-quality oil, vitamins, and particular antioxidant components like sesamol and sesamin, as well as high protein content (Mujtaba et al., 2020; Liang et al., 2021). Sesame meal is a good source of phosphorus and nitrogen, which makes it a good fertilizer and raises the amount of organic matter in the soil (Ma & Fang, 2019). However, sesame productivity is impacted by climatic variables (Ibrahim, 2015). In addition, the narrow genetic base of sesame poses a problem in breeding programs (Ju et al., 2021). Increasing plant diversity would increase the area devoted to sesame cultivation in Egypt (Shabana et al., 2014; Anter, 2020; Sahab et al., 2021).

Hence, to select stable and high-yielding lines, it was necessary to evaluate several new lines of sesame using the greatly modified multi-environment trials

(METs) (Khan et al., 2020; Olanrewaju et al., 2021). However, interactions between genotypes and environmental interaction factors (GEI) make it challenging to adopt the genotypes recommended by breeders (Olanrewaju et al., 2021; Bakare et al., 2022). There are many methods to avoid this problem, such as principal component analysis (PCA), additive main effects and multiplicative interactions (AMMI = the additive main effects and multiplicative interaction), and GGE biplot analysis (GGE biplot = genotype + genotype x environment). The AMMI tool determines the effect of genotypes and environmental conditions as additive effects and their interactions as a multiplicative component. As variation components, the principal component analysis will then be used (Gauch, 2013). Since molecular markers are unaffected by environmental factors, they are used with morphological criteria for identifying cultivars effectively, studying genetic variability, and protecting plant breeders' rights (Ibrar et al., 2022; Samaha et al., 2023; Adly et al., 2023). RAPD and ISSR markers provide an efficient means of identifying the cultivars, estimating polymorphism, and studying the genetic relationships among different genotypes and have been used for diversity analysis of many crop species, including sesame (Mawcha et al., 2020; Anter & Samaha, 2021; El-Badan et al., 2022, 2024; Nasser et al., 2024; Youssef et al., 2024).

The present study aims to evaluate 28 advanced sesame lines in 23 seed production environments over six growing seasons to identify high-yielding

and stable lines to obtain climate-ready cultivars. RAPD and ISSR markers were used to detect DNA polymorphisms and the relationship between sesame genotypes.

MATERIALS AND METHODS

Plant materials

The breeding materials used in this study were elite-derived sesame lines (Table1) obtained by pedigree selection from the Department of Agronomy, Faculty of Agriculture, Cairo University (Saber, 2015). The following are the genotypes' names: C1 (cv. Shandweel), C2 (cv. Sohag), 3 (C1.5), 4(C1.6), 5 (C1.8), 6 (C1.9), 7 (C2.2), 8 (C2.3), 9 (C2.6), 10 (C3.4), 11 (C3.7), 12 (C3.8), 13 (C5.7), 14 (C5.8), 15 (C6.2), 16 (C6.3), 17 (C6.4), 18 (C6.5), 19 (C6.7), 20 (C6.9), 21 (C6.11), 22 (C6.12), 23 (C8.4), 24 (C8.8), 25 (C8.11), 26 (C9.3), 27 (C9.6), 28 (C9.7), 29 (C9.15) and 30 (C9.20). Commercial varieties were C1 (Shandawil) and C2 (Sohag), obtained from the Ministry of Agriculture and Land Reclamation. The genotypes were evaluated in field trials over six consecutive cropping seasons (2018 - 2023). The field trials materialized in five sites (Al Sharqia, Bani Suef, Giza, Menoufia, and Nubaria) as shown in Table 2. The experimental sites represent different climatic conditions, giving 23 environments (location-year combinations).

Experimental design

The plot consists of three rows, each 3.0 m in length, with 60 cm between-row spacing and 10cm in-row spacing. All the experiments were carried out in a randomized complete block design (RCBD) with three replications. The yield (t/ha) was calculated from a net area of 5.4 m². The recommended agronomic procedures were followed.

Statistical analysis

The studied trait was subjected to analysis of variance (ANOVA) to estimate the differences found between genotypes, locations, seasons, genotype by location, genotype by season, and genotype by location by season using MSTAT-C. After detecting the presence of the interaction, phenotypic stability analysis was made using the AMMI model. AMMI can detect GEI in a multi-dimensional environment and display it using a biplot. The GGE biplots graphical image was used for genotype ranking based on mean and stability (Yan & Tinker, 2006).

Genomic DNA extraction and PCR conditions

The DNA extraction process was conducted by the

genomic DNA isolation kit "GeneDireX, Inc." according to the manufacturer's instructions from sesame leaves. Eight RAPD primers and 10 ISSR primers were selected for PCR amplification. PCR reactions contain 1 µL DNA (20 ng/µL), 1 µL primer (10 µM) (ISSR / RAPD), 6.3µL of COSMO PCR RED Master Mix (Cat. No.WF10203001, Willowfort, Birmingham), and ddH₂O up to 12.5 µL. A PCR program for DNA amplification: initial denaturation at 94 °C for 4 min, then 35 cycles of denaturation at 94 °C for 1 min, annealing at 37 °C for RAPD primers and 40 -58°C for ISSR primers for 45 s, and extension at 72 °C for 2 min, with a final extension at 72 °C for 10 min. The PCR product was analyzed in a 1.5% agarose gel in 1X TBE buffer along with the BERUS 100 bp DNA ladder (WF10407001, Willowfort, Birmingham). The profiles were documented using a UV transilluminator.

Molecular data analysis

The software GelAnalyzer v. 19.1 was utilized to examine the gel images. Polymorphic information content (PIC) was determined using the online program PICcalc (<http://w3.georgikon.hu/pic/english/default.aspx>) according to Nagy et al. (2012). Estimating similarities was done using Jaccard's coefficient. The NTSYS-pc, v. 2.1 package (Rohlf, 2000) was used for clusteranalysis.

RESULTS AND DISCUSSION

Variance and performance of genotypes

Increasing the supply of crops is one of the most important strategies to combat the negative impacts of climate change. The current study evaluated some new sesame lines based on seed yield in five locations according to the results in Table 3. The results showed significant differences ($p \leq 0.05$) in seed production across all individual experiments. It indicates that there is genetic variation, which is helpful in the selection processes for sesame improvement. These findings agreed with Baraki & Gebremariam (2018), Anter & El-Sayed (2020) and Movahedi et al. (2020). The coefficient of variation values was less than 20% in all individual experiments, which indicated that the experiments conducted with a high degree of accuracy, according to Lopes et al. (2021). This variation is reflected in the average performance of sesame genotypes regarding seed yield (t/ha) across twenty-three environments. Fifteen lines exceeded C1 and C2 in seed yield, namely C9.6, C2.6, C2.2, C5.8, C9.3, C8.11, C9.20, C3.8, C6.3, C5.7, C6.11, C1.6, C6.9, C6.12 and C6.2.

Table 1. Source of new sesame lines according to their breeding status and parents' traits.

parents	Breeding Status	Source of seeds *	Specific traits
P1 (HM19)	F ₈ -hybrid pop	Cairo Univ.*	Early maturity, non-branching first capsule set low, 3 capsules/axil.
P2 (EUL90)	Mutant line	Cairo Univ.*	Early maturity, non-branching, first capsule set low, 3 capsule3/axil.
P3 (Mutant48)	Mutant line	Cairo Univ.*	Branching, 3 capsules /axil.
P4 (Giza 32)	Local variety	Ministry of Agric.& Land Reclamation, Egypt	Heavy seed weight, medium branching, one capsule/axil, long capsule, late maturity, resistance of <i>Fusarium Oxysporum</i> is moderate
P5 (NM59)	Exotic line	India through IAEA**	Stiff stem, late maturity, one capsule/axil.
P6 (Babil)	Exotic variety	Iraq through IAEA**	Low branching, 3 capsules/axil, semi- shattering capsules.
C1 (Shandaweel)	Local cultivar	Ministry of Agric.& Land Reclamation, Egypt	White seed, length mean, 3 capsules/axil, a non-branching variety
C2 (Sohag)	Local cultivar	Ministry of Agric.& Land Reclamation, Egypt	Brown seed, late maturity,3 capsules/axil, a non-branching variety

* Advanced breeding materials resulted from the breeding program conducted at Agron. Dept. Fac. Of Agric. Cairo Univ. * * Inter. Atomic Energy Agency.

Table 2. Characterization of the study sites (Abbreviations: OMC: Organic matter content)

		Bani Suef	Giza	Nubaria	Al Sharqia	Menoufia
Geographical description	Latitude (N)	29° 4' 0" N	29.9870°N	30° 36' 36" N	30° 42' 0" N	30°33'51"N
	Longitude (E)	31° 5' 0" E	31.2118°E	30° 25' 48" E	31° 37' 48" E	31°09'26"E
Soil properties	PH	8.1	8.3	8.60	8.02	8.1
	Texture %	Clay	Clay	Sandy	Clay	Clay
	OMC %	1.44	1.12	0.38	1.85	1.90
Temperature means						
May		28.0	37.1	25.2	29.3	27.0
Jun		30.5	39.4	27.8	31.4	27.8
Jul		31.1	35.5	28.8	31.4	30.2
Aug		31.1	36.7	28.9	31.9	30.7
Sep		28.8	36.8	26.7	30.2	28.5

This superiority may be due to the new genotypes have undergone various changes, including changes in genome size, structural chromosome rearrangements, and gene expression (Rao et al., 2009; Bird et al., 2018; Azzam et al., 2021; Khaled et al., 2022; Azzam et al., 2023). When we evaluated the lines in comparison with their constituent parents and commercial varieties, we found that the lines were longer in terms of root length, plant height, and number of capsules, which was reflected positively on the final yield (Abdelraouf & Anter 2020; Anter et al., 2024; Mehanna et al., 2024). In addition, their interaction with the environments helped the lines achieve higher physiological and behavioral responses to different environments compared to their parents. This agreed with earlier studies by Anastasi et al. (2017) and Baraki et al. (2020).

Concerning the environments, E1 (Clay soils) provided the highest average seed yield of 1.11 t/ha. In contrast, E22 (sandy soil) had the lowest average seed yield (0.600 t/ha). Clay soils recorded the highest yield because they contained the largest and most active terrestrial carbon, which allows plants to produce more photosynthesis, resulting in higher yields (Evans, 2013). In addition to the differences between the soils in terms of organic matter, this indicates that sesame seed yield was highly affected by the different environments (Nadeem et al., 2015; Baraki et al., 2020). Given that the response of the genotypes differs from one site to another over the years because of the presence of interaction, a combined analysis was conducted to determine the main effects and their interactions for the source of variations as seen in Table 4. The mean square of the source

Table 3. Mean performance and variance of sesame genotypes for seed yield (t/ha) across 23 environments (E) (Abbreviations: \bar{X} : Means. CV: Coefficients of variation. LSD: Least significant difference. **: Significant level at 0.05)

Location Gen./Envi.	Al Sharqia (2019-2021)				Bani Suef (2019-2023)							
	E1	E2	E3	E4	E5	E6	E7	E8	E9	E10	E11	E12
C1	1.076	1.175	1.125	1.010	0.980	0.750	0.700	0.970	0.970	0.826	0.720	1.050
C2	1.215	0.725	0.971	0.900	0.960	0.700	0.693	0.863	0.888	0.700	0.396	0.900
C1.5	1.100	0.600	0.851	0.875	1.000	0.750	0.750	1.250	0.925	0.791	0.900	0.950
C1.6	1.250	1.100	1.175	1.250	1.000	0.750	1.250	1.000	1.050	0.840	0.981	1.000
C1.8	1.250	0.900	1.076	0.975	0.875	0.625	0.750	0.875	0.820	1.036	0.945	1.100
C1.9	0.900	0.851	0.876	0.438	0.500	0.375	0.375	0.625	0.463	0.396	0.450	0.630
C2.2	1.250	1.100	1.175	1.125	1.063	1.625	0.500	2.250	1.313	0.749	1.035	1.100
C2.3	1.001	0.975	0.989	0.619	0.584	0.894	0.275	1.238	0.722	0.412	0.569	0.605
C2.6	1.151	1.076	1.113	0.938	0.925	0.500	0.625	0.675	0.733	0.875	1.260	1.050
C3.4	1.001	1.425	1.214	0.516	0.509	0.275	0.344	0.371	0.403	0.481	0.693	0.578
C3.7	1.250	1.100	1.175	0.250	0.250	0.875	1.000	1.875	0.850	0.875	1.008	0.950
C3.8	1.250	0.900	1.076	0.750	1.500	1.250	0.750	1.250	1.100	0.826	1.080	0.950
C5.7	1.226	1.001	1.113	1.250	0.750	0.625	1.125	1.000	0.950	0.910	1.080	1.050
C5.8	1.001	0.975	0.989	0.750	0.750	1.000	1.000	1.000	0.900	0.791	1.071	0.975
C6.2	1.151	1.151	1.151	1.125	1.250	0.750	1.250	1.000	1.075	1.029	0.990	0.400
C6.3	0.900	0.851	0.876	0.750	1.125	1.000	0.750	0.700	0.865	0.763	0.324	1.075
C6.4	1.200	1.350	1.275	1.050	0.875	0.925	1.125	0.550	0.905	0.770	0.810	0.750
C6.5	1.200	1.350	1.275	0.750	1.575	0.813	0.750	0.750	0.928	0.791	0.540	0.900
C6.7	1.250	1.100	1.175	0.250	0.750	1.000	0.375	0.595	0.595	0.924	0.315	1.150
C6.9	1.001	0.950	0.975	0.500	0.750	1.125	1.075	0.625	0.815	0.980	0.405	1.150
C6.11	1.275	1.001	1.139	1.300	1.750	0.825	1.293	1.058	1.245	0.763	0.495	0.400
C6.12	1.001	0.851	0.926	0.750	0.625	1.000	1.575	1.375	1.065	0.826	0.540	0.975
C8.4	1.100	0.600	0.851	0.538	0.538	0.663	0.463	0.525	0.545	0.403	0.540	0.538
C8.8	0.575	0.776	0.675	1.075	1.075	1.325	0.925	1.050	1.090	0.805	1.080	1.075
C8.11	1.500	0.851	1.176	0.975	0.875	0.700	0.825	0.808	0.808	0.945	1.035	1.000
C9.3	1.001	0.926	0.963	1.250	1.750	1.875	1.550	1.925	1.670	0.749	0.630	1.150
C9.6	1.250	1.301	1.275	2.400	1.300	2.300	1.800	1.950	1.950	0.777	0.450	0.650
C9.7	1.001	1.151	1.076	0.825	1.250	0.875	0.750	0.875	0.915	0.819	0.720	1.000
C9.15	0.900	1.100	1.001	0.375	0.750	1.000	0.375	0.625	0.625	0.784	0.540	0.750
C9.20	1.125	1.226	1.176	1.750	0.250	1.000	0.625	0.908	0.908	0.924	0.495	1.050
\bar{X}	1.112	1.015	1.063	0.910	0.938	0.939	0.855	1.019	0.936	0.785	0.737	0.897
SL 0.05	**	**	**	**	**	**	**	**	**	**	**	**
CV%	6.8	7.6	16.0	15.0	16.1	12.1	10.1	11.2	10.3	12.1	16.7	17.1
LSD	0.115	0.139	0.518	0.529	0.583	0.538	0.383	0.251	0.258	0.348	0.522	0.528
Location Gen./Envi.	Giza (2018-2021)				Nubria (2019-2022)						Menoufia (2022)	
	E13	E14	E15	E16	E17	E18	E19	E20	E21	E22	E23	\bar{X}
C1	0.980	0.790	0.780	1.000	1.100	0.900	0.970	0.900	0.780	0.480	0.999	0.914
C2	0.890	0.700	0.765	0.970	0.890	0.860	0.900	0.930	0.990	0.690	0.743	0.836
C1.5	0.750	1.275	0.405	0.915	0.975	0.470	0.816	0.693	1.095	0.800	1.200	0.875
C1.6	0.750	0.600	0.600	0.840	0.800	0.929	0.576	0.804	0.570	0.270	0.900	0.882
C1.8	0.900	0.625	0.525	0.875	0.750	0.410	0.456	0.483	1.029	0.730	0.900	0.822
C1.9	0.675	0.474	0.468	0.744	0.837	0.710	0.677	0.435	0.926	0.630	0.720	0.616
C2.2	1.000	0.500	0.450	0.840	0.625	1.151	0.768	1.011	0.510	0.210	1.001	0.972
C2.3	0.550	0.275	0.248	0.462	0.344	0.588	0.422	0.556	0.281	0.300	0.550	0.585
C2.6	0.900	1.350	0.855	1.180	1.390	0.980	1.296	1.188	1.251	0.950	1.430	1.030
C3.4	0.495	0.743	0.470	0.649	0.765	0.494	0.713	0.653	0.688	0.390	0.786	0.637
C3.7	1.000	0.700	0.420	0.840	0.700	0.659	0.504	0.630	1.080	0.780	1.136	0.866
C3.8	0.750	0.700	0.966	1.165	1.475	1.232	0.336	0.834	0.759	0.460	1.217	0.982
C5.7	0.900	0.500	0.560	0.740	0.500	1.040	0.444	0.792	1.332	1.030	1.200	0.918
C5.8	0.850	1.000	0.990	1.320	1.725	0.614	1.320	1.017	1.140	0.840	1.151	1.007
C6.2	0.975	0.640	0.500	0.615	0.545	1.079	1.008	1.095	0.714	0.410	0.534	0.889
C6.3	1.200	0.600	0.975	1.125	1.115	1.088	1.440	1.314	1.179	0.880	1.230	0.962
C6.4	0.800	0.500	0.495	0.720	0.665	0.566	0.432	0.549	1.188	0.890	0.675	0.829
C6.5	0.500	0.700	0.420	0.700	0.700	0.971	0.360	0.717	0.666	0.370	0.750	0.803
C6.7	1.150	0.575	0.405	0.890	0.625	1.241	0.336	0.840	1.071	0.770	1.200	0.808
C6.9	1.150	1.200	1.350	1.440	1.725	1.352	0.864	1.158	0.966	0.670	1.151	1.016
C6.11	0.800	0.500	1.125	1.145	1.690	0.971	0.432	0.753	1.200	0.900	0.974	1.001
C6.12	0.600	0.885	0.600	0.865	0.945	0.263	1.416	0.891	1.071	0.770	0.783	0.896
C8.4	0.325	0.255	0.375	0.435	0.440	0.172	0.792	0.533	0.182	0.400	0.360	0.503
C8.8	0.650	0.510	0.750	0.870	0.880	0.443	1.584	1.065	0.363	0.430	0.720	0.860
C8.11	1.050	0.800	0.850	0.800	0.750	1.328	0.890	0.834	0.860	0.820	0.900	0.930
C9.3	1.050	0.500	0.675	0.955	0.815	1.109	0.690	0.951	0.675	0.380	1.050	1.056
C9.6	0.850	0.500	0.600	0.625	0.500	1.034	0.834	0.984	0.600	0.300	0.974	1.096
C9.7	0.500	0.500	0.420	0.675	0.600	1.328	1.128	1.278	0.660	0.360	1.125	0.862
C9.15	0.900	0.500	0.570	0.775	0.725	0.524	0.624	0.624	0.975	0.680	0.984	0.726
C9.20	1.125	1.300	0.495	1.075	1.065	1.058	1.584	1.371	0.624	0.320	1.175	0.984
\bar{X}	0.834	0.690	0.637	0.875	0.889	0.852	0.820	0.863	0.848	0.600	0.951	0.872
SL 0.05	**	**	**	**	**	**	**	**	**	**	**	-
CV%	12.9	8.9	25.0	17.2	7.2	9.3	8.7	12.0	17.1	19.8	18.1	-
LSD	0.323	0.165	0.981	0.630	0.194	0.166	0.148	0.118	0.161	0.148	0.158	-

variance showed significant differences ($p \leq 0.05$) for seed yield. Genotypes by years (G x Y), genotypes by locations (G x L), and genotypes by locations by years (G * L * Y) had significant differences in seed yield, which indicated that the performance of genotypes was not consistent across different environments. It is called crossover performance (Baraki et al., 2020; Tohidi et al., 2020; Khaled et al., 2023).

AMMI analysis

Highly significant differences in years, locations, and genotypes may be due to the changes in environmental conditions and the genetic makeup, which varies in performance from one environment to another. However, the analysis of variance does not provide details of the G x E interactions. Therefore, multivariate analysis, stability parameters, AMMI, and GGE biplot are commonly used in describing G x E interaction and estimating the stability of genotypes over different environments (Oladosu et al., 2017; Khan et al., 2021; Anter et al., 2024). In Table 5, seed yield was analyzed using AMMI ANOVA and by examining the genotypes through experiments at multiple sites. According to the AMMI G * E seed production model, which divided the components into six major components, significant differences among the parts of G, E, G * E, and IPC parts were found (Baraki & Gebremariam, 2018; Baraki et al., 2020). The cumulative contribution of IPC1 and IPC6 justified 90.4% of the total interactions, demonstrating the efficiency of the AMMI in determining interaction (Baraki & Gebremariam, 2018; Movahedi et al., 2020).

Furthermore, the AMMI biplot was applied to demonstrate that the impact of each genotype and environment value IPCA1 closest to the axis start point has less effect on interaction than those farther away. PC1 and PC2 together explain 68.38% of the total variance and confirm the strong influence of G * E on production seed yield (Figure 1). The five lines C5.8, C3.8, C6.11, C6.2, and C9.6 are the most stable because these genotypes were close to the center of the two-plot and arrowhead parent. At the same time, they exceed in producing seed yield and can be classified as ideal lines. Most environments are on the right side of the biplot because the genotypes were assessed under normal conditions. On the other hand, the E15 environment contributed to the G x E interaction because it is located on the left side. Coordinated homogeneity of lines C6.2, C1.9, C3.8, and C5.8 with eighteen

environments located in all test sites because they formed an acute angle with sites, indicating that they were more adapted and stable compared to the other genotypes, as indicated by Frutos et al. (2014) and Movahedi et al. (2020). In summary, according to the average seed production performance of the lines and the GGE biplot, lines C5.8, C6.11, C9.6, C6.2, C2.2, C6.4, C2.6, C1.9, and C3.8 can be considered as new sources that can be used in the sesame breeding program, to develop new varieties that combine high seed productivity and stability to face the adverse effects of climate change.

RAPD analysis

A total of 78 amplified bands were generated from eight primers ranging in size from 140 to 1500 bp, with an average number of bands per primer of 9.8 as displayed in Table 6. The total number of polymorphic bands was 63, ranging from four (OP-C05 and OP-A20) to 16 (OP-C01), averaging 7.9 bands. Polymorphism percentages ranged from 55.56% (OP-A18) to 94.12% (OP-C01), with a mean of 77.5%, revealing a high level of polymorphism in sesame. Idris et al. (2023) reported similar results, observing 75% polymorphism in sesame. However, this percentage was greater than 58.48% (Kumar & Sharma, 2009) and 60.42% (Anter & Samaha, 2021). Three primers generated eight unique bands, covering seven genotypes; primer OP-A08 produced two bands for C1 (560 bp) and C2.2 (1290 bp), and four bands for C8.4 (140 and 280 bp), C2.6 (170 bp), and C3.4 (230 bp) obtained using primer OP-C01. Two unique bands were produced using primer OP-C09 for C2.3 (243 bp) and C6.12 (312 bp). RAPD primers revealed three markers associated with seed yield: two positive markers, OP-A07 (440 bp), OP-A20 (300 bp), and one negative marker, OP-A07 (900 bp). In addition, there are two markers associated to the yield stability trait: one positive OP-C09 (210 bp) and one negative OP-A08 (630 bp). PIC values ranged from 0.3578 to 0.4996, with a mean of 0.43. All primers were moderately informative for polymorphism. The PIC value was (0.43) higher than the previous RAPD studies (0.186 and 0.40) reported by Dar et al. (2017) and Saha et al. (2019), respectively. RAPD profile produced by the primer OP-C01 is shown (Figure 2).

ISSR analysis

Of 10 ISSR primers, 84 bands were produced, with an average of 8.4 bands per primer. Based on the findings in Table 6, the number of bands varied from 5 to 12, with a size of 150 to 1590 bp.

Table 4. Combined analysis of variance for sesame genotypes for seed yield (t/ha) over 23 environments (Abbreviations: CV: Coefficients of variation. LSD: Least significant difference. **: Significant level at 0.05)

Source of variance	Degree of freedom(D.F.)	Mean square(MS)
Replication	2	4193.0**
Locations (L)	22	810.0**
Years (Y)	5	558.0**
L*Y	110	906.0**
Genotype (G)	29	980.0**
G*L	638	976.0**
G*Y	145	994.0**
G*L*Y	3190	1115.0**
Error	8004	1000
CV %	14.7	
LSD 0.05	0.290	

Table 5. AMMI analysis of variance of seed yield (t/ha) of sesame genotypes (Abbreviations: IPCA: Integrated principal components analysis. **: Significant level at 0.05)

Source	Degrees of freedom(DF)	Sum of squares(SS)	Mean square(MS)
Total	2069	57349269.0	27718.0
Treatments	689	57347429.0	83233**
Genotypes	29	6357832.0	219236**
Environments	22	27434483.0	1247022.0**
Block	46	1840.0	40.0
Interactions	638	23555114.0	36920.0
IPCA	50	10083677.0	201674.0**
IPCA	48	4457154.0	92857.0**
IPCA	46	3393722.0	73777.0**
IPCA	44	1617638.0	36765.0**
IPCA	42	974237.0	23196.0**
IPCA	40	799987.0	20000.0**
Residuals	368	2228699.0	6056
Error	1334	-	-

Table 6. List of RAPD and ISSR primers, their sequences, and amplification results (Abbreviations: Tm °C: Melting temperature. PIC: Polymorphic information content)

Primer name	Primer sequence(5'-3')	Tm °C	Molecular weight range (bp)	Total number of amplified bands	Number of monomorphic bands	Number of polymorphic bands	Polymorphism %	PIC
RAPD								
OP-A07	GTGACGTAGG	32	216-1176	9	2	7	77.78	0.3410
OP-A08	AGGTGACCGT	34	297-1345	13	1	12	92.30	0.4947
OP-A18	GTTGCGATCC	32	203-1409	9	4	5	55.56	0.4996
OP-A20	CCTTGACGCA	32	187-953	7	3	4	57.14	0.3578
OP-B12	TTCGAGCCAG	34	285-932	8	1	7	87.50	0.4025
OP-C01	GATGACCGCC	32	140-1500	17	1	16	94.12	0.4520
OP-C05	CTCACCGTCC	34	273-1053	6	2	4	66.67	0.3962
OP-C09	GAAACGGGTG	34	179-1357	9	1	8	88.89	0.4930
Total		-	-	78	15	63	-	-
Mean		-	-	9.8	1.9	7.9	77.5	0.43
ISSR								
UBC-815	CTCTCTCTCTCTCTG	52.0	460-1171	7	2	5	71.43	0.4819
UBC-825	ACACACACACACACT	50.4	276-1250	9	4	5	55.56	0.3018
UBC-826	ACACACACACACACC	52.8	150-942	8	2	6	75.00	0.4875
UBC-834	AGAGAGAGAGAGAGYTT	54.0	273-875	5	3	2	40.00	0.1128
UBC-835	AGAGAGAGAGAGAGATC	55.0	191-690	5	3	2	40.00	0.0263
UBC-847	CACACACACACACARC	55.02	231-1590	9	3	6	66.67	0.3768
UBC-855	ACACACACACACACAYT	52.5	243-1033	9	6	3	33.33	0.1049
UBC-864	ATGATGATGATGATGATG	43.0	487-1377	11	3	8	72.73	0.3795
UBC-868	GAAGAAGAAGAAGAAGAA	47.0	453-1487	12	2	10	83.33	0.4548
UBC-881	GGGTGGGGTGGGGTG	54.0	211-1415	9	3	6	66.67	0.4842
Total		-	-	84	31	53	-	-
Mean		-	-	8.4	3.1	5.3	60.5	0.32

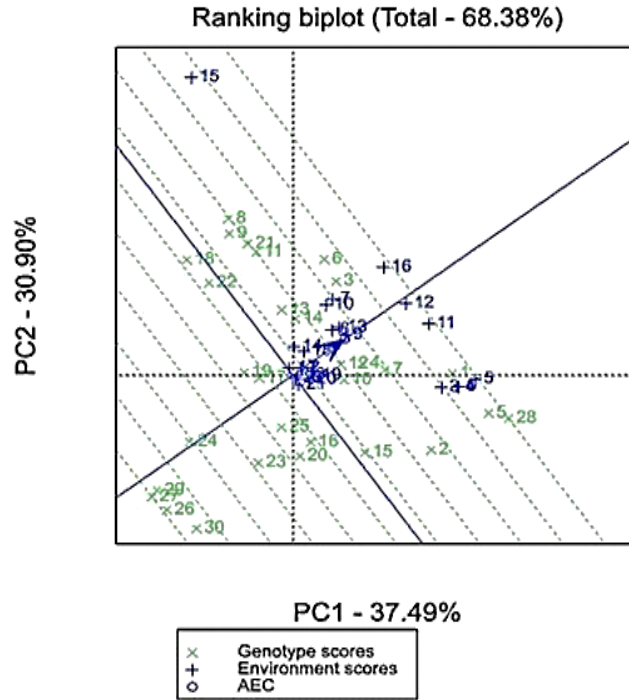


Figure 1. AMMI2 biplot displaying the first two main axes of interaction for seed yield (IPCA2 vs. IPCA1), x-coordinate = the main effects (means) and the y-coordinate = the interaction

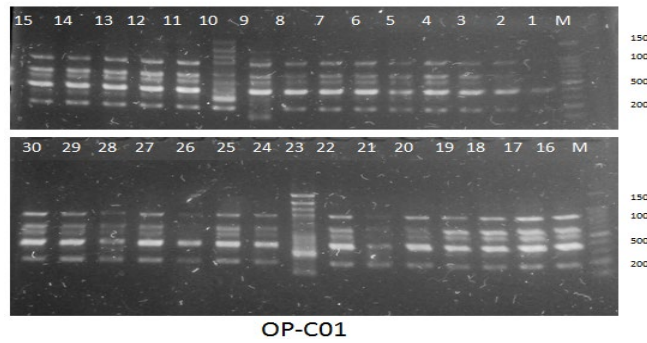


Figure 2. RAPD profile of the 30 sesame genotypes generated by primer OP-C01.

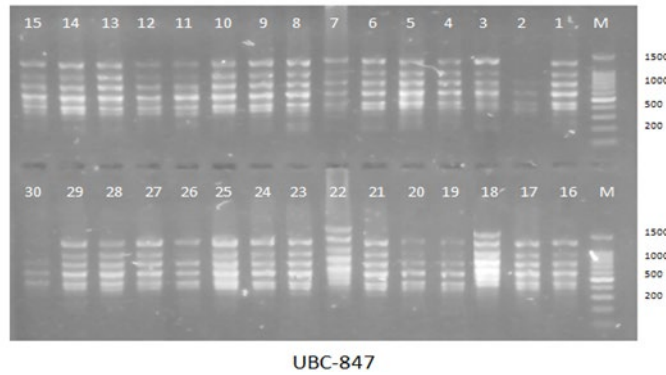


Figure 3. ISSR profile of the 30 sesame genotypes generated by primer UBC-847.

The total number of polymorphic bands was 53, averaging 5.3 per primer. The UBC-868 primer exhibited the highest polymorphic band (10), while UBC-834 and UBC-835 produced the lowest bands (2). Polymorphism percentages ranged from 33.33% (UBC-855) to 83.33% (UBC-868), with a mean of 60.47%, which was greater than 57% and 47.17%, as reported by Kumar & Sharma (2011) and Anter & Samaha (2021), respectively. However, this percentage was lower than the 89.61% and 80.7% reported by Singh et al. (2015) and El Harfi et al. (2021). Only three primers successfully amplified five unique bands in four genotypes. UBC-815 yielded two unique bands of sizes 460 and 500 bp for C6.7 and C9.20, respectively. Furthermore, the UBC-847 marker revealed two unique bands of size 1540 and 1590 bp for C6.12 and C6.5, respectively. UBC-868 produced a unique band of 780 bp for C6.7. The ISSR markers revealed two positive markers with molecular size 170bp for UBC-826 and 220bp for UBC-834 related to seed yield and yield stability, respectively. PIC values ranged from 0.1049 to 0.4875, with a mean of 0.32. Seven primers showed moderate polymorphism, with a PIC \geq 0.25. This PIC value was lower than the values published by Abate et al. (2015) and Patel et al. (2016), which were 0.43 and 0.67, respectively, and higher than the 0.19 reported by El Harfi et al. (2021). ISSR profile produced by the primer UBC-847 is shown (Figure 3).

Previous results showed that the RAPD markers revealed higher genetic variability than ISSR markers among sesame genotypes, as confirmed by Patel et al. (2016). On the other hand, this finding contrasts with the result of Singh et al. (2015), who found that ISSR has more polymorphism than RAPD in sesame. This difference in polymorphism may be the result of somatic mutation leading to the genetic variation as observed by Zhang et al. (2008) or variation in the type and number of primers, the genotypes used environments, and their interaction (Aboelnaga et al., 2020; El Harfi et al., 2021). The high PIC value of a marker is one of the most important indicators to measure genetic diversity accurately (Dutta et al., 2016). Therefore, the use of RAPD primers (OP-A08, OP-A18, OP-C01, and OP-C09) and ISSR primers (UBC-815, UBC-826, UBC-868, and UBC-881) were the most informative for measuring genetic diversity in sesame.

Genetic relationships and cluster analysis

The genetic similarity among the thirty sesame genotypes based on RAPD markers ranged from 0.72

(between Sohag and C8.4) to 0.97 (between C9.6 and C9.15). The dendrogram obtained from RAPD markers showed the division of sesame genotypes into four clusters (Figure 4a). Cluster 1 consisted of Shandweel and Sohag cultivars. Clusters 2 and 3 each had one line, C8.4 and C1.5, respectively, forming a distinct operational taxonomic unit (OTU). Cluster 4 contained the other genotypes. Similarly, RAPD was used to determine the genetic similarity among sesame, which ranged from 0.510 to 0.885, as described by Dar et al. (2017).

Based on ISSR markers, the genetic similarity varied between 0.83 (Sohag and C8.11) to 0.98 (C2.2 and C2.3). The dendrogram showed four clusters: C9.3 and C9.6 lines in cluster 1, C8.11 line in cluster 2, C9.20 and Sohag in cluster 3, and the remaining genotypes in cluster 4 (Figure 4b). A similar result by Gebru et al. (2019) found that the genetic similarity among sesames ranged between 0.56 to 0.82.

The similarity coefficients of the sesame genotypes based on the combined data of RAPD + ISSR markers ranged from 0.81 (between Sohag and C8.11) to 0.94 (between C5.7 and C6.2). Four major clusters could be identified on the dendrogram (Figure 4c). The two commercial cultivars (Shandweel and Sohag) are in cluster 1. C8.11 line in cluster 2, 6 lines in cluster 3, and 21 lines in cluster 4.

The results indicated that the closest relationships were between the genotypes (C9.6 and C9.15), (C2.2 and C2.3), and (C5.7 and C6.2), revealing a high level of similarity, which may be due to their release from the same parental origins. Conversely, the C8.4, C8.11, and C1.5 lines showed significant genetic differences compared to the other lines. Perhaps these relationships reflect the ancestry and biology of the genotypes (Metais et al., 2000).

CONCLUSIONS

The current study evaluated 28 advanced sesame lines in 23 environments as new plant genetic resources to counteract climate change and maintain biodiversity. The study identified 15 lines with higher yields than the controls, of which five were the most stable and could be recommended for experimental yields and as parents in sesame breeding programs. The study also noted that RAPD and ISSR markers could be valuable in revealing the genetic variations in sesame and identifying markers associated with phenotypic traits. Finally, to improve sesame breeding, we could use genetically distant lines or create crossbreeding program that combines

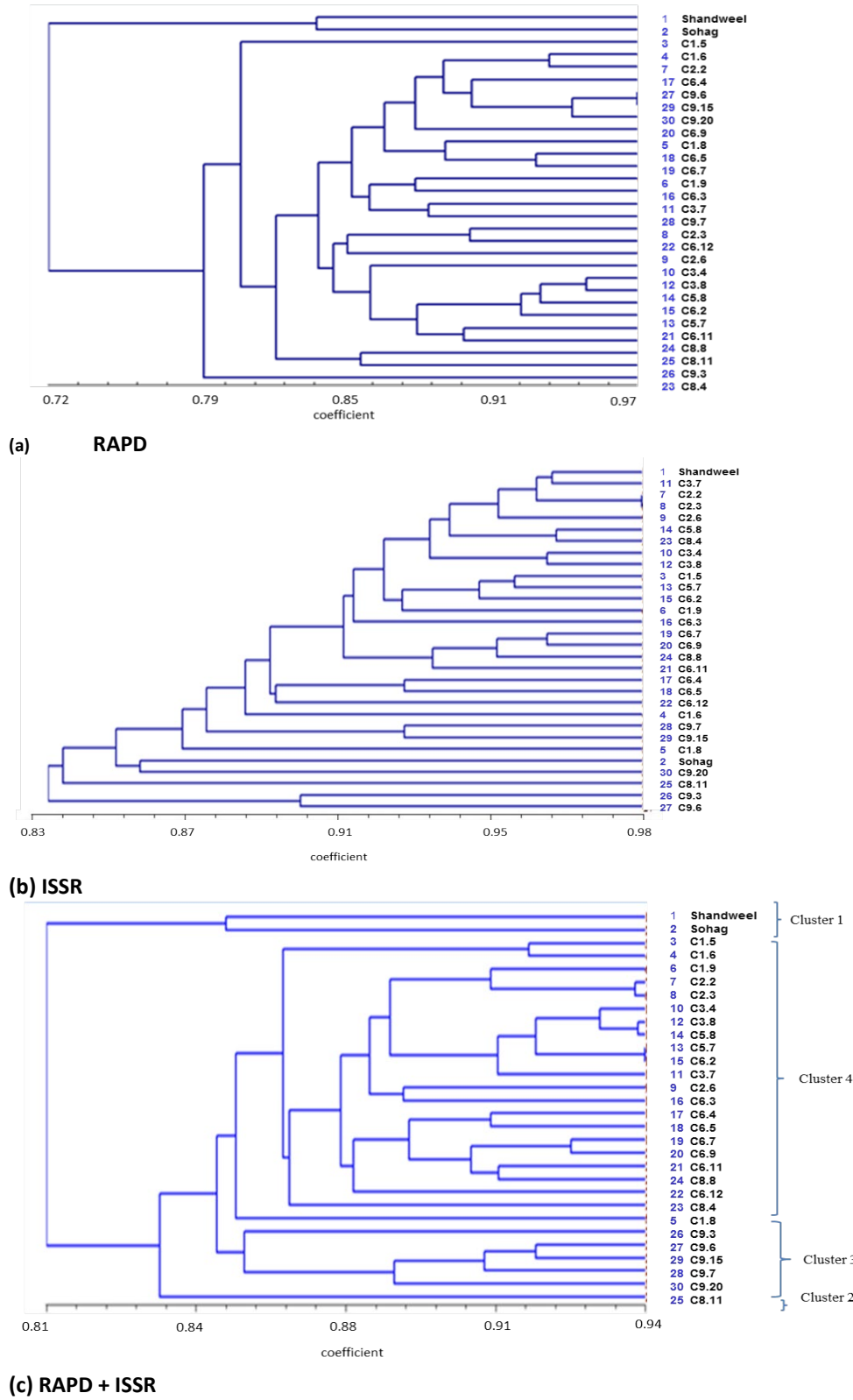


Figure 4. UPGMA dendrograms among 30 sesame genotypes were generated using data obtained by (a): RAPD, (b): ISSR, and (c): RAPD + ISSR markers, respectively.

parents with high yields with parents that are more resilient to climate change.

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COMPETING INTERESTS

The authors report no conflicts of interest regarding this work.

AUTHORS' CONTRIBUTIONS

GS: molecular markers, data collection, a compilation of literature sources, data analysis, assessment, interpretation, writing the article, and preparation of the final draft. AA: identifying plant species, field experiments, data collection, compilation of literature sources, data analysis, assessment, interpretation, writing the article, and preparation of the final draft. SM: providing the laboratory equipment.

ETHICS APPROVAL

Not applicable

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