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Evaluation of the growth, yield traits and the genetic diversity of some *Brassica napus* genotypes under Egyptian conditions

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Abstract

Background Canola (B*rassica napus* L.) is considered an alternate oilseed plant. Therefore, this study aimed to evaluate some growth parameters, yield, chemical parameters and genetic diversity among thirteen canola genotypes including a collection of Chinese, German, French, and local genotypes under Egyptian conditions.

Result Trapper genotype recorded the highest values of plant height (47.12 and 89.75 cm) and dry weight/plant (8.54 and 28.19 dry weight/plant) at 60 and 90 days from sowing, respectively. Data from the field experiments showed that significant differences were recorded among tested genotypes for all yield and its component parameters (i.e., plant height (cm), branches/no. plant, siliquas and seed weight (g/plant) and seed oil %. The genetic diversity and the relationships among the thirteen canola genotypes were evaluated utilizing sequence-related amplified polymorphism (SRAP) and simple-sequence repeats (SSRs) markers. The allelic frequency of the different SRAP and SSR markers tested has differed among the thirteen canola genotypes. The SRAP and SSR analyses showed 659 out of 742 and 15 out of 45 markers, respectively, were detected as polymorphic markers (88.8% and 33.33%) among the tested wheat cultivars In addition, the polymorphism information content (PIC), marker index (MI) and resolving power (RP) parameters were computed to assess the effectiveness of the markers. The results indicated the occurrence of a considerable genetic variation between Chinese, European and Egyptian genotypes.

Conclusion These markers are of considerable value and can be utilized to screen large canola populations. The results of the comparison between the two molecular markers showed that the most effective marker that showed the genetic diversity between canola genotypes was SRAP (88.8%) polymorphism. It could be concluded that the tested canola genotypes could be cultivated under the Egyptian condition with high performance especially Trapper, Agamax and Topas genotypes. Therefore, it could be suggested that these three genotypes seem to be promising for oil gap reduction and need further evaluation for the expansion under new reclaimed regions.

Keywords Genetic diversity, Growth parameters, SRAP, SSR, *Brassica napus* L

1 Background

There is a large gap between edible oil production and consumption in Egypt; therefore, high amount are imported from abroad. For that, the government policy is to rely on *B. napus* L crops. The *Brassica* genus contains about 100 species, including *B. napus* L. that known as canola or rapeseed [1]. Canola is an amphidiploid (2n = 4 X = 38) generated by hybridization between *B. rapa* and *B. oleracea* [2]. Recently, there are agricultural expansions to increase canola production in Egypt. Canola considers

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the third most substantial source of edible oil after soybean and palm in the world. Canola seeds contain 42% oil and 25% protein [3]. Although *B. napus* oil is considered an important source of vegetable oil, the level of erucic acid and glucosinolate in the seed may limit its usage [4].

The knowledge of the genetic relationship and diversity between genotypes is important [5]. Canola genotypes were classified into spring, winter and semi-winter genotypes [6]. Hybridization between these genotypes is an important approach to developing the genetic base of canola genotypes [7]. The morphological parameters, protein content, isozymes and DNA markers were utilized to assess the genetic relationship between plant genotypes. The positive relationship between number of pods, seeds/pod and 1000—seed weight with seeds/plant and quality of some canola genotypes were reported by other studies [8, 9]. The positive relationship between number of pods, seeds/pod and 1000—seed weight with seeds/plant and quality of some canola genotypes were reported [9, 10]. Isozymes have been utilized as markers in many genetic studies including genetic variation in *B. juncea* [11]. However, morphological and chemical parameters are affected by the plant developmental stage and surrounding environmental conditions. Among several markers used for genetic analysis, DNA markers are more effective, specific and reliable in discriminating closely related genotypes [12–14]. Several molecular markers, including restriction fragment length polymorphism (RFLP), amplified fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), sequence characterized amplified regions (SACR), single nucleotide polymorphisms (SNP), SRAP and SSR, have been utilized to map genes and study the genetic diversity among different canola genotypes [15, 16]. SRAP DNA marker was mentioned first time by Li and Quiros [17]. SRAP combines two primers, each containing a random sequence with CCGG sequence in the forward primer and AATT in the reverse one, and amplifies the polymorphism related to the open reading frames (ORFs) [18]. SRAP and SSR markers are reliable, simple, highly polymorphic, easily detected and generally utilized in genomic applications [19, 20]. In comparison with other marker types, SSR markers are codominant, reproducible and relatively inexpensive when the primer sequence is known. Moreover, SSR often occurs in gene-rich genome sequences, increasing their possible relevance for quantitative trait loci studies. SSR markers have been widely utilized in genetic diversity studies in wheat, maize, rice and tomatoes [13, 21, 22]. Cunmin et al., [24] and Ahmad et al., [25] displayed that the SSR marker was efficient for genetic variation evaluation among different canola genotypes. Therefore, this study aims to evaluate the growth parameters, yield and yield attributes and some chemical constituents among thirteen canola genotypes collected from different regions under Egyptian conditions. In addition, to study the genetic diversity of these canola genotypes utilizing SRAP and SSR markers.

2 Methods

2.1 Plant material

Thirteen canola genotypes were collected from German, China and France to cultivate with two local genotypes under the Egyptian condition (Table 1). The experiment was cultivated in the Agricultural production and research station, National research center (NRC), El Nubaria, El Behira governorate, Egypt during two seasons (2019–2020 and 2020–2021). The experimental soil texture was sand. Soil chemical and physical properties were evaluated utilizing Chapman and Pratt [26] method (Table 2). Irrigation water was analyzed (Table 3).

2.2 Sowing

The soil was plowed two times and divided into plots. 200 kg/ha calcium superphosphate and 100 kg/ha potassium sulfate were added during seed preparation, while four equal doses from ammonium sulfate (160 kg/ha) were applied weekly. Each plot contains fifteen rows (about 20 cm spacing) of 3.5-m length, i.e., 10.5 m2, with a seed rate of 8 kg/ha. The planting date for the first and second season was the 20^{th} and 25^{th} of November, respectively. Sprinkler irrigation took place immediately after sowing, then every seven days at intervals according to agronomic practices in the district.

2.3 Growth characters

After sowing five plants were randomly selected at 60 and 90 days from each plot to assess the plant height (cm), the

Table 1 Canola genotype name and origin

Genotype code	Genotype name	Origin	Reference
1	Serw 4	Egypt	[27]
2	HE you 46	China	[28]
3	Pactol	France	[27]
4	RG 4605	German	[29]
5	Semu DNK 234/84	German	[29]
6	HE you 56	China	[28]
7	Serw 6	Egypt	[30]
8	Wang you 25	China	[28]
9	Semu DNK 65/84	German	[29]
10	AD 201	German	[29]
11	Topas	French	[29]
12	Agamax	German	[31]
13	Trapper	German	[31]

Table 2 Chemical and physical analysis of the experimental soil (average of both seasons)

Seasons	Physical analysis									Chemical analysis							
	Depth (cm)	Sand %	Silt %	Clay %	Soil texture	pH	EC dS/m	$CaCO_3$	Organic Matter %	Soluble cations meq/l					Soluble anions meq/l		
										Na^+	K^+	Mg^{++}	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	$SO4^{--}$
1	0–30	88.97	8.30	2.73	Sandy	7.54	0.50	11.15	0.14	0.79	0.28	2.92	2.49	0.12	1.42	3.11	2.67
2	30- 60	89.51	7.61	2.88	Sandy	8.20	0.70	11.32	0.25	0.86	0.41	2.75	2.67	0.14	1.39	3.18	2.18

Table 3 Chemical analysis of irrigation water (average of both seasons)

Characters	pH	EC dS/m	Soluble cations meq/l					Soluble anions meq/l		
			Na^+	K^+	Mg^{++}	Ca^{++}	CO_3^{--}	HCO_3^-	Cl^-	$SO4^{--}$
	7.6	4.00	30.10	0.77	4.15	14.24	0.07	3.57	31.90	4.92

number of leaves/plants, and the number of branches/plants, the fresh and dry weight/plant (g).

2.4 Yield and yield attributes

Ten plants were collected randomly at the harvest to assess, plant height, number of siliqua/plant, 1000-seed weight (g), and seed yield/plant (g), while seed, straw and biological yield/ha (kg/ha) were determined by harvest all area of the plot.

2.5 Chemical analysis

Potassium, phosphate, iron, manganese and zinc contents were measured in the digested samples utilizing a Jenway flame photometer [32]. The dried plants were then completely ground to a fine powder and total N, P, K, Fe, Mn and Zn were measured according to A.O.A.C. [33]. Seed protein content was calculated by multiplying N (%) by 5.75. Seed oil content was assessed by utilizing Soxhlet apparatus and petroleum ether 60–80 °C as a solvent as described by A.O.A.C. [33]. The photosynthetic pigments (chlorophyll a, b and total carotenoids) were determined in representative fresh leaves samples after 60 and 90 days from sowing using spectrophotometer (Jasco, serial No.C317961148, Japan) at the wavelength of 663 nm for chlorophyll a, 647 nm for chlorophyll b and 470 nm for total carotenoids. The chlorophyll a and chlorophyll b were measured according to Moran and Porath [34] as follows: ten ml *N*,*N*-Dimethylformamide (DMF) was added to 1 g of fresh leaves in dark tubes; then, they were left for overnight at 5 °C. The obtained extracts from previous materials were measured spectrophotometrically while the DMF was used as a blank. The total carotenoids were determined by the method of Yang et al. [35]. 1 g of fresh leaves was mixed with 10 mL of an acetone–hexane mixture (2:3) for 2 min. The absorbance maxima were read at 470 nm for carotenoids.

The concentrations of these pigments were calculated employing formula [34, 35]:

$$\text{Chlorophyll a} = 12.76\ A_{663} - 2.79\ A_{647}\ (\text{mg/l})$$

$$\text{Chlorophyll b} = 20.76\ A_{647} - 4.62\ A_{663}\ (\text{mg/l})$$

$$\text{Total chlorophyll} = 17.90\ A_{647} - 8.08\ A_{663}\ (\text{mg/l})$$

$$\text{Total carotenoids} = \left(1000 \times A_{470} - 2.72 \times \text{chlorophyll a} - 81.4 \times \text{chlorophyll b}\right)/227\ (\text{mg/l})$$

All the above were calculated on the fresh weight basis as mg/g fresh leaves.

2.6 DNA extraction

In the second experimental season, chromosomal DNA was extracted from the thirteen canola genotypes utilizing the cetyltrimethylammonium bromide (CTAB) method [36].

Table 4 The nucleotide sequences of SRAP primers

Forward Primer	Sequence (5′–3′)	Reverse primer	Sequence (5′–3′)
ME1	TGAGTCCAAACCGGATA	EM1	GACTGCGTACGAATTAAT
ME2	TGAGTCCAAACCGGAGC	EM2	ACTGCGTACGAATTTGC
ME3	TGAGTCCAAACCGGAAT	EM3	GACTGCGTACGAATTGAC
ME4	TGAGTCCAAACCGGACC	EM4	ACTGCGTACGAATTTGA
ME5	TGAGTCCAAACCGGAAG	EM5	GACTGCGTACGAATTAAC
ME7	TGAGTCCAAACCGGTAA	EM6	GACTGCGTACGAATTGCA
ME8	TGAGTCCAAACCGGTGC	EM9	GACTGCGTACGAATTCAA
ME9	TGAGTCCAAACCGGAAT		

2.7 SRAP analysis

Polymerase chain reaction (PCR) of SRAP was carried out in a 25 µL reaction volume including 2 µL DNA (50 ng/µL), 12.5 master mix (GeneDireX® One PCR™, Cat. No. SM213-0250, Taiwan), 1 µL (2 µM/µL), from forward and reverse primers and 8.5 µL of nuclease-free water. Fifteen SRAP primers were utilized in this investigation (Table 4). PCR conditions were programed with a denaturation step at 94 °C for 5 min, followed by 5 cycles at 94 °C for 1 min, then annealing at 35 °C for 1 min, and extension at 72 °C for 1 min, followed by 35 cycles of denaturation at 94 °C for 1 min, and annealing step at 50 °C for 1 min, then extension step at 72 °C for 1 min. Finally, the amplification was completed with one cycle of a final extension at 72 °C for 7 min.

2.8 SSR analysis

Twenty SSR primers were utilized to study the genetic variation among canola genotypes (Table 5). PCR analysis was performed in a 10 µl reaction mixture containing 1 µl DNA (50 ng/µL), 5 µL master mix (GeneDireX® One PCR™, Cat. No. SM213-0250, Taiwan), 0.5 µL (2 µM/µL) from each primer and 3 µL of dH_2O. The PCR program started with a denaturation step at 94 °C for 5 min, followed by 35 cycles at 94 °C/1 min, for denaturation, and annealing changed according to each primer (Table 5), then elongation step at 72 °C/1 min and, finally, a terminal extension step at 72 °C/5 min.

For both SRAP and SSR, the amplification conditions were performed in a thermocycler UNO II, Biometra, Germany. The products were resolved by 2% agarose gel in 1 × TAE buffer, DNA bands visualized with ethidium bromide staining (0.5 µg/mL), and photographed under UV light using the gel documentation system (Bio-Rad® Gel Doc-2000). Fifty bp DNA ladder (GeneDireX®, Cat. No. G DM012-R500, Taiwan) was used as a molecular weight size marker.

2.9 Statistical analyses

The analysis of the collected data was carried out utilizing the least significant difference (LSD) test at 0.05 levels. For data obtained each season, the results were analyzed utilizing the analysis of variance of significance according to Gomez and Gomez [37]. The SRAP and SSR products were scored based on the presence (1) or absence (0) of the bands. The data obtained SRAP and SSR analyses were collected together to determine the genetic similarity coefficient between samples utilizing the Dice coefficient [38]. PIC, MI, and RP parameters were obtained for each primer following Chesnokov and Artemyeva [39] to calculate the in formativeness of the tested primers.

Table 5 The nucleotide sequences and the annealing temperature of the SSR primers

No	Primer name	Forward (5′–3′)	Reverse (5′–3′)	Annealing Temp.℃
1	BRMS01 (GA)25	GGTGGCTCTAATTCCTCTGA	ATCTTTCTCTCACCAACCCC	53
2	BRMS02 (CT)22	GATCTTCTCTCCAAAA	TCCAAGCTAAATTACG	45
3	3 BRMS03 (CT)19 F	ACGAATTGAATTGGACAGAG	CAGATGGGAGTCAAGTCAAC	51
4	A049627743	ATGGAATCTGCTCATCTCAC	TAAGCTGCAATGATCAAAGAT	49
5	A0415440685	TTTGAACGATACACAACAACA	GTTGGTCCACGAGTAAAAGAT	52
6	A05 20,242,013	AGAAGCTTTTTCTCTTGTTGC	TGATGTAAGAGCGTGAAAGAT	50
7	A01-21,437,996	GAATACATGGAGAGATCTGGA	CATTTAGAAATCAGAGCGAAG	51
8	BG 103	TTTGTCCACCATTTCTTAAACATCTA	TCAATGAAATTGTTAAAATACAGCAA	53
9	BG 1	GCTGGCTGCACAATAACAGA	TACCACTGGAGGAGCTTCG	55
10	BG 55	TCCCAAGTGGTGCTCCTTT	GGTAATCCCTTTTCTGCAAGC	53
11	BRMS56 (GA)13	GATCAAGGCTACGGAGAGAGAG	CGTGACGCTAGAGTAATCGAGT	58
12	BRMS17 (CA)33	GGAAAGGGAAGCTTCATATC	CTGGAAAGCATACACTTTGG	51
13	SR1	GTTTGGTTCAGAGGCAGAGG	CTATCGCTGCAGAAGAAGGG	55
14	BJ 96	GATCTTCTCTCCAAAACTCTCT	AAAGTCCAAGCTAAATTACAAA	50
15	BJ 95	CGTAAGTTTCAATTGTCAACGG	TCGTACGAAACAATCAACGG	52
16	IM 4	CCCAAACGCTTTTGACACAT	GGCACAATCCACTCAGCTTT	52
17	IM 8	GCGATGTTTTTTCTTCAGTGTC	TTAATCCCTACCCACAATTTCC	53
18	SR 37	TATGTACACATTCCTCATTTTC	CATTCGTCTCCACCTTCT	49
19	BRMS19 (GA)10	CCCAAACGCTTTTGACACAT	GGCACAATCCACTCAGCTTT	52
20	BRMS27 (GA)17	GTGCTTGATGAGTTTCACATTG	GCAGGCGTTGCCTTTATGTA	53

Table 6 Growth characters of 13 canola genotypes after 60 and 90 days (D) from sowing

	Plant height		No. of leaves/plant		No. of branches/plant		Fresh weight (g)/plant		Dry weight (g)/plant	
	60D	90D	60D	90D	60D	90D	60D	90D	60D	90D
Serw 4	39.54	76.11	16.32	19.50	4.32	8.29	41.25	102.82	7.69	26.54
HE you 46	32.26	63.00	16.12	19.18	5.28	9.25	41.12	102.51	6.11	23.46
Pactol	44.12	84.35	17.45	21.32	5.12	9.09	39.15	97.76	8.25	27.63
RG 4605	44.44	74.48	13.57	20.27	2.83	8.87	16.27	101.03	4.26	25.87
Semu DNK 234/84	44.25	76.31	13.58	20.24	2.84	9.09	16.35	103.52	4.28	26.26
HE you 56	33.54	65.31	17.15	20.84	4.99	7.96	38.98	97.35	6.38	23.99
Serw 6	38.29	73.86	16.55	19.87	3.68	7.65	38.12	95.28	7.54	26.25
Wang you 25	37.25	71.99	16.28	19.44	4.57	8.54	37.15	92.94	7.12	25.43
Semu DNK 65/84	42.80	73.16	13.44	19.18	2.76	7.15	15.67	89.24	4.11	21.59
AD 201	38.19	73.68	16.87	20.39	4.36	8.33	42.23	105.19	7.94	27.03
Topas	42.12	80.75	17.54	21.49	5.84	9.81	40.58	101.21	7.49	26.15
Agamax	44.65	84.59	17.65	21.64	4.68	8.65	45.12	112.15	8.15	27.44
Trapper	47.12	89.75	16.99	20.58	4.12	8.09	42.32	105.40	8.54	28.19
LSD	2.11	3.15	0.64	1.21	1.12	0.95	3.11	3.67	1.21	2.11

3 Results

3.1 Growth characters

The evaluation of thirteen canola genotypes grown under Egyptian conditions and study their growth characteristics, i.e., plant height, number of leaves/plants, number of branches/plants, fresh weight/plant, dry weight/plant, chlorophyll a, chlorophyll b, and total carotene after 60 and 90 days from sowing were displayed in Table 6. The results displayed that all studied characters showed significant differences in both seasons except for chlorophyll b and total carotene after 90 days from sowing.

Data presented in Table 6 illustrated the growth characteristics of canola genotypes cultivated under the Egyptian condition. The results displayed that Trapper genotype surpassed in plant height and dry weight/plant; however, it recorded 47.12, 89.75 cm and 8.54, 28.19 g for plant height and dry weight/plant at 60 and 90 days after sowing, respectively. While the Agamax genotype surpassed it in both sampling times in the number of leaves/plant, fresh weight/plant and chlorophyll b where it recorded 17.65, 21.64, 45.12, 112.15 g and 1.01, 1.57 for the number of leaves/plant, fresh weight/plant and

Table 7 Total pigment of 13 canola genotypes after 60 and 90 days (D) from sowing

	Chlorophyll a		Chlorophyll b		Carotene		Total	
	60D	90D	60D	90D	60D	90D	60D	90D
Serw 4	0.38	0.63	0.99	1.54	0.26	0.27	1.63	2.44
HE you 46	1.34	1.25	0.79	1.20	0.92	0.97	3.05	3.42
Pactol	1.80	1.53	0.77	1.17	1.24	1.30	3.81	3.5
RG 4605	1.55	0.97	0.47	1.30	0.31	0.84	2.35	3.12
Semu DNK 234/84	1.56	0.99	0.47	1.33	0.32	0.86	2.36	3.19
HE you 56	1.40	1.35	0.67	1.00	0.96	1.01	3.03	3.36
Serw 6	0.25	0.42	0.67	1.00	0.17	0.18	1.09	1.6
Wang you 25	0.49	0.82	0.81	1.23	0.33	0.35	1.63	2.4
Semu DNK 65/84	1.50	0.95	0.45	1.08	0.30	0.83	2.26	3.06
AD 201	0.36	0.60	0.81	1.23	0.24	0.26	1.41	2.09
Topas	0.60	1.01	0.92	1.42	0.41	0.43	1.93	2.86
Agamax	0.42	0.70	1.01	1.57	0.28	0.30	1.71	2.57
Trapper	0.38	0.63	0.94	1.45	0.26	0.27	1.58	2.35
LSD	0.49	0.54	0.33	NS	0.58	NS	0.62	0.66

chlorophyll b at 60 and 90 days after sowing, respectively. Topas genotype surpassed in the number of branches/plants in both sampling times where it records 5.84 and 9.81 for 60 and 90 days after sowing, respectively. Pactol genotype surpassed in chlorophyll a, carotene and total pigment in both sampling times where it recorded 1.80 and 1.53, 1.24 and 1.30, 3.81 and 3.5 for chlorophyll a, carotene and total pigment at 60 and 90 days after sowing, respectively (Table 7). While the HE you 46 genotype recorded the lowest value of plant height, Semu DNK 65/84 genotype recorded the lowest values of the number of leaves/plant, number of branches/plant, fresh weight/plant, dry weight/plant and chlorophyll b and Serw 6 genotype recorded the lowest values of chlorophyll b, carotene and total pigments at 60 and 90 days after sowing, respectively.

3.2 Yield and yield attributes

Data in Table 8 illustrated that yield and yield attributes, i.e., pod number/m^2, 1000 seed weight, straw yield/faddan, pod yield/faddan, seed yield/faddan and biological yield/faddan of 13 canola genotypes cultivated under the Egyptian conditions, where the recorded characters differed significantly between the tested genotypes except 1000 seed weight. Trapper genotype surpassed the other genotypes in the recorded parameters except biological yield/faddan, where it recorded 783.59, 3.52, 4850.20, 3946.61, 1972.21 and 7087.06 for pod number/m^2, 1000

Table 8 Yield and yield attributes of 13 canola genotypes

Genotypes	Pod number/m^2	1000 seed weight	Straw yield/faddan	Pod yield/faddan	Seed yield/faddan	Biological yield/faddan
Serw 4	708.77	3.30	4389.80	3731.87	1803.79	6942.75
HE you 46	736.51	3.31	4558.81	3709.51	1853.73	6661.79
Pactol	695.16	3.13	4326.63	3631.26	1755.28	6629.10
RG 4605	699.21	3.18	4336.57	3617.06	1768.18	6609.65
Semu DNK 234/84	727.17	3.30	4510.04	3761.74	1838.90	6874.04
HE you 56	666.19	3.10	4126.07	3507.67	1695.42	6520.08
Serw 6	771.43	3.35	4747.00	3584.98	1915.75	7142.47
Wang you 25	725.09	3.15	4461.81	3369.60	1800.66	6707.64
Semu DNK 65/84	713.19	3.24	4423.31	3689.40	1803.54	6741.84
AD 201	652.84	2.94	4063.22	3410.18	1648.42	6225.52
Topas	691.67	3.11	4281.27	3483.67	1740.87	6256.22
Agamax	739.59	3.33	4603.18	3863.36	1867.47	7052.82
Trapper	783.59	3.52	4850.20	3946.61	1972.21	7087.06
LSD	15.32	NS	65.23	35.12	44.51	34.15

Table 9 Chemical characters of 13 canola genotypes seed

Genotypes	Protein %	Oil %	N %	P %	K %	Fe ppm	Mn ppm	Zn ppm
Serw 4	25.52	44.68	4.43	0.53	0.93	67.57	12.33	25.18
HE you 46	24.84	46.35	4.32	0.49	1.01	94.58	15.51	28.97
Pactol	23.43	46.51	4.07	0.55	0.96	80.05	14.72	28.68
RG 4605	23.31	45.17	4.05	0.45	0.93	88.57	13.68	25.54
Semu DNK 234/84	23.77	46.07	4.13	0.46	0.94	90.34	13.95	26.05
HE you 56	23.99	45.98	4.17	0.42	0.96	84.14	14.30	24.03
Serw 6	24.42	45.18	4.24	0.52	0.95	83.14	12.71	24.83
Wang you 25	22.95	47.12	3.99	0.45	0.94	103.87	14.88	29.34
Semu DNK 65/84	24.24	46.97	4.21	0.47	0.96	92.11	14.25	26.56
AD 201	22.00	45.65	3.82	0.48	0.85	43.53	11.89	23.09
Topas	23.33	44.98	4.05	0.49	1.00	49.96	14.01	25.13
Agamax	24.93	46.12	4.33	0.43	0.97	66.76	13.44	27.48
Trapper	26.43	45.85	4.59	0.52	1.01	76.76	13.58	27.36

seed weight, straw yield/faddan, pod yield/faddan, seed yield/faddan and biological yield/faddan, respectively. Serw 6 recorded the second order after Trapper in most of the studied characters except pod yield/faddan and biological yield/faddan, while AD 201 genotype recorded the lowest values of the studied characters.

3.3 Chemical characters

Data in Table 9 illustrated that some chemical constituents, i.e., protein %, oil %, N %, P %, K %, Fe ppm, Mn ppm and Zn ppm of 13 canola genotypes cultivated under the Egyptian conditions, where Trapper genotype surpassed the other genotypes in protein %, N % and K %, while Semu DNK 65/84 genotype surpassed in oil %. Pactol genotype surpassed P% while the HE you 46 genotypes surpassed in Fe and Mn and Wang you 25 genotypes surpassed in Zn content.

3.4 The genetic diversity among canola genotypes using SRAP marker

From 56 SRAP primer combinations, only 36 combinations generated suitable polymorphism in the 13 tested canola genotypes (Fig. 1). SRAP analysis resulted in a total of 742 bands detected among the thirteen canola genotypes. Only 659 of them were polymorphic bands (88.8%) (Table 10). The highest number of bands (34 bands) was generated by using both (EM4-ME7and EM9-ME2) primers, while the lowest one was 10 bands that were generated with EM1-ME7.The (EM3-ME9, EM3-ME7, EM3-ME8, EM6-ME1, EM6-ME2, EM1-ME7, EM3-ME3, EM4-ME1, EM9-ME8, EM4-ME7, EM9-ME2, EM9-ME3 and EM9-ME7) markers recorded the highest polymorphism percentages (100%) while the lowest one was 6 bands that were generated with percentage (23.07%) was recorded by EM6-ME3 marker. The unique band of SRAP marker for some genotypes of canola was determined (Table 11). Furthermore, the parameters of the genetic varieties for the investigated primers were determined. The polymorphism information content (PIC) values ranged from (0.107) obtained with primer EM6-ME4 and (0.390) by EM9-ME4. In addition, the marker index (MI) values indicated range from (0.197) by primers EM3-ME8 and (4.195) by EM6-ME4. Also, the calculated resolving power (RP) values were ranged between (5.17) by EM3-ME8 and (26.83) by EM6-ME4.

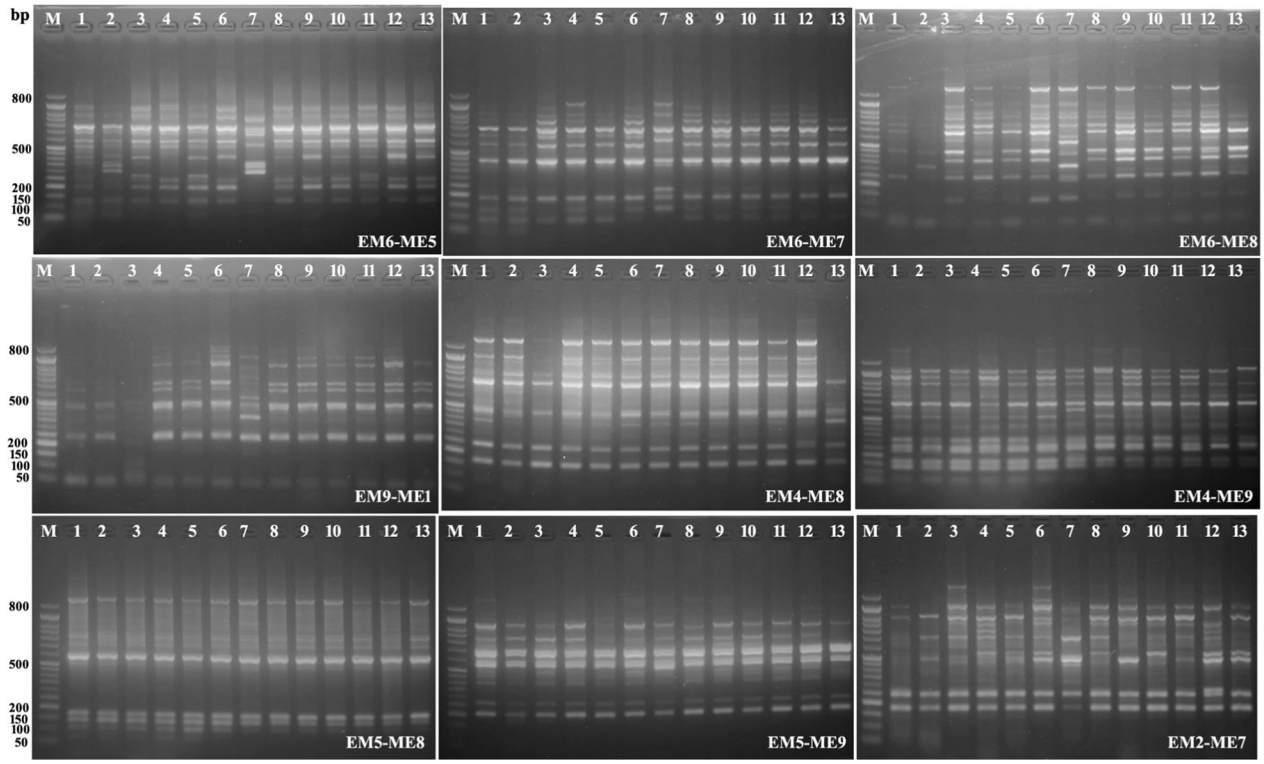

Fig. 1 SRAP profile demonstrating polymorphism among the canola genotypes. M refers to 50 bp DNA ladder marker. Lanes 1–13 represent all the canola genotypes

Table 10 PCR amplicons obtained from SRAP markers

Primer name	Total bands	Polymorphic band	Polymorphism %	PIC	MI	RP
EM1-ME8	13	11	84.6	0.223	2.080	15.33
EM1-ME9	18	16	88.80	0.361	0.968	10.00
EM2-ME7	20	18	90	0.353	1.408	13.67
EM2-ME8	20	18	90	0.360	1.601	14.83
EM2-ME9	22	21	95.45	0.311	2.293	18.33
EM3-ME9	18	18	100	0.259	0.475	6.83
EM3-ME7	30	30	100	0.341	0.716	9.50
EM4-ME7	34	34	100	0.262	0.371	5.33
EM5-ME7	13	9	69.23	0.149	1.384	12.17
EM6-ME9	15	12	80	0.224	2.546	18.50
EM9-ME7	20	20	100	0.222	1.047	9.00
EM6-ME3	13	3	23.07	0.022	3.669	22.00
EM9-ME2	34	34	100	0.268	0.832	9.17
EM9-ME3	29	29	100	0.352	0.778	10.00
EM9-ME4	28	27	96.42	0.390	1.156	12.50
EM9-ME5	23	21	91.3	0.330	2.564	19.83
EM9-ME8	27	27	100	0.361	1.383	13.50
EM9-ME9	23	22	95.65	0.300	1.218	12.00
EM3-ME8	30	30	100	0.287	0.197	5.17
EM4-ME8	19	18	94.7	0.264	1.885	14.67
EM4-ME9	19	12	63.19	0.254	3.00	21.83
EM5-ME8	17	9	52.94	0.155	2.768	18.83
EM5-ME9	19	12	63.15	0.199	2.288	16.67
EM6-ME1	24	24	100	0.363	1.434	13.83
EM6-ME2	26	26	100	0.360	0.635	9.33
EM6-ME4	17	6	35.29	0.107	4.195	26.83
EM6-ME5	23	22	95.65	0.159	2.298	16.33
EM6-ME7	20	17	85	0.254	2.015	16.00
EM6-ME8	22	19	86.36	0.267	1.853	15.33
EM9-ME1	19	16	84.21	0.229	2.064	15.83
EM1-ME7	10	10	100	0.357	1.281	11.17
EM2-ME4	15	11	73.3	0.256	1.124	16.50
EM2-ME5	17	14	82.35	0.339	2.249	19.00
EM3-ME3	14	14	100	0.362	1.248	12.50
EM3-ME5	12	10	83.33	0.300	1.390	12.00
EM4-ME1	19	19	100	0.284	0.605	7.17
Total	742	659	88.8			

3.5 The genetics similarity based on SRAP analysis

The result of genetic similarity based on SRAP analysis indicated the highest number was (0.70) between cultivar (AD 201 and Topas) while the lowest number was (0.34) between cultivar (Serw 6 and Serw 4) and (Serw 6 and HE you 46) (Additional file 1: Table S1). The dice similarity index classified the canola genotypes into two main clusters. The first one included only Serw 6 genotype while the second cluster has divided into 2 subclusters; the first included Serw 4 (1) genotypes and HE you 46 (2) genotype and the second has divided into another 2 subclusters. One of them included Trapper and Agamax and the other one also divided into two subclusters (Additional file 1: Fig. S1). The dendrogram showed that genotypes Topas and AD201 were in the same cluster.

3.6 SSR analysis

SSR analysis resulted in a total of 45 bands detected among the thirteen canola species (Fig. 2). Only 15 of them were polymorphic bands (33.33%). The highest number of

Table 11 Canola genotypes unique band of SRAP marker

Canola genotypes	Positive	Negative	Total
Serw 4	17(E3M7,E4M7,E6M9,E9M7,E6M3,E3M 8,E5M8,E9M4,E2M4,E4M1)	3(E2M7, E6M2, E6M8)	20
HE you 46	7(E3M9,E3M7,E4M7,E6M9,E9M7,E5M8,E6M7)	7(E9M7, E6M5, E6M8, E9M8)	14
Pactol	8(E1M9,E3M7,E5M7,E9M1,E9M4,E9M9)	1(E9M1)	9
RG 4605	4(E3M7, E5M7, E6M5)	0	4
Semu DNK 234/84	3(E3M7, E4M7, E5M9)	0	3
HE you 56	4(E4M1, E9M7, E3M8, E9M3)	0	4
Serw 6	46(E1M8,E1M9,E2M7,E2M8,E2M9,E5M7,E6M2,E9M7,E9M3,E9M8,E3M3,E3M 8,E4M9,E6M1,E6M2,E6M5,E6M7,E9M1,E9M2,E9M5,E9M9,E3M5)	23(E1M8,E6M9,E6M5,E6M7,E6M8,E9M1 ,E9M2,E9M5,E9M8,E2M4)	69
Wang you 25	2(E3M7, E5M9)	1(E5M9)	3
Semu DNK 65/84	3(E1M9, E3M7)	1(E3M5)	4
AD 201	1(E4M7)	0	1
Topas	4(E3M8, E9M3, E9M4)	5(E9M9, E9M4)	9
Agamax	19(E4M1,E2M7,E2M8,E3M9,E4M7,E5M9,E9M2,E9M3)	4(E1M9, E3M9, E9M2)	23
Trapper	5(E2M5, E4M7, E4M2, E9M3)	5(E3M9,E4M9,E5M9,E6M4,E9M2)	10
Total	123	50	173

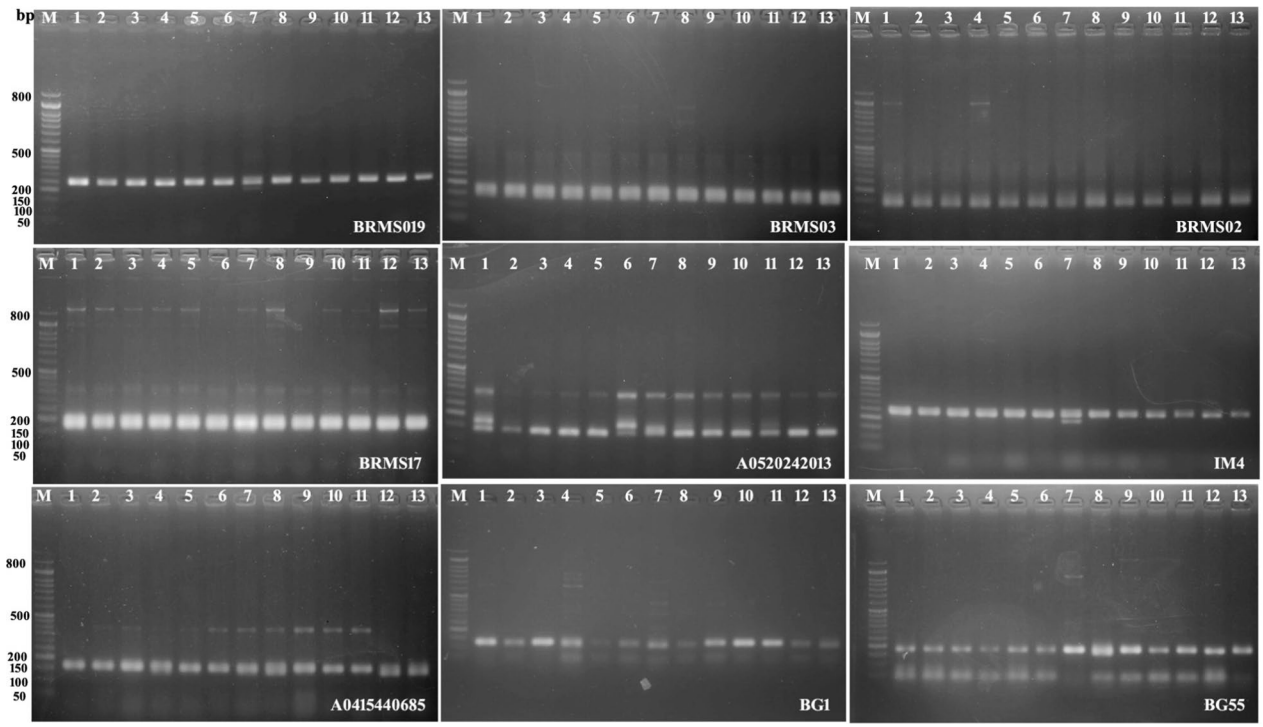

Fig. 2 SSR profile demonstrating polymorphism among the canola genotypes. M refers to 50 bp DNA ladder marker. Lanes 1–13 represent all the canola genotypes

bands (3 bands) was amplified by using (A049627743, A0415440685, A05 20,242,013, A01-21,437,996, BG 103, BJ 95, IM 4 and SR 37) primers while the lowest one was one band that was generated with primers (BRMS02 (CT) 22, SR1 and BJ 96). The highest polymorphism percentages (66.67%) were recorded by primers (A0415440685, A05 20,242,013, BG 103, BJ 96 and SR 37) while the lowest polymorphism percentages (0%) was recorded by primer (BRMS02 (CT)22, 3 BRMS03 (CT)19, BG 1, BRMS56 (GA)13, BRMS17 (CA) 33, SR1 and BJ 95) (Table 12). Further, the PIC values were range from (0.00) by 9 primers (BRMS56(GA)13, BRMS17(CA)33,

Table 12 PCR amplicons obtained from SSR markers

Primer name	Total bands	Polymorphic band	Polymorphism %	PIC	MI	RP
BRMS01 (GA)25	2	1	50	0.076	0.141	3.83
BRMS02 (CT)22	1	0	0	0.00	0.00	2.00
3 BRMS03 (CT)19 F	2	0	0	0.00	0.00	4.00
A049627743	3	1	33.3	0.051	0.145	5.83
A0415440685	3	2	66.67	0.176	0.276	3.67
A05 20,242,013	3	2	66.67	0.199	0.388	4.50
A01-21,437,996	3	1	33.3	0.051	0.145	5.83
BG 103	3	2	66.67	0.310	0.502	4.17
BG 1	2	0	0	0.00	0.00	4.00
BG 55	2	1	50	0.139	0.235	3.67
BRMS56 (GA)13	2	0	0	0.00	0.00	4.00
BRMS17 (CA)33	2	0	0	0.00	0.00	4.00
SR1	1	0	0	0.00	0.00	2.00
BJ 96	2	1	50	0.139	0.235	3.67
BJ 95	3	0	0	0.00	0.00	6.00
IM 4	3	1	33.3	0.051	0.102	4.17
IM 8	2	0	0	0.00	0.00	4.00
SR 37	3	2	66.67	0.176	0.423	5.33
BRMS19 (GA)10	2	1	50	0.076	0.077	2.17
BRMS27 (GA)17	1	0	0	0.00	0.00	2.00
Total	45	15	33.33			

Table 13 Canola genotypes unique band of SRR marker

Canola genotype	Positive	Negative	Total
Serw 6	3 (IM4, Bg55, Primer 3)	2 (Bj95, Bg55)	5
Semu DNK 65/84	0	1 (Ga 25)	1
Trapper	0	2 (SR37, Primer 1)	2
Total	3	5	8

BRMS02(CA)22, BG1, 3BRMS03(CA)19F, SR1, BJ95, IM8 and BRMS27(GA)17) to (0.310) by primer BG103. Moreover, the highest MI value (0.502) was gained by primer BG103, while the lowest value (0.00) was obtained with the same 9 primers for the PIC. Further, the calculated RP values were about (6.00) by primer BJ95 to (2.00) by three primers (BRMS27(GA)17, SR1 and BRMS02(CT)22). The unique band of SSR marker for some genotypes of canola was determined (Table 13).

3.7 The genetics similarity based on SSR analysis

The highest number of genetic similarity based on SSR analysis was (1.00) between genotype (Pactol and Semu DNK 65/84) and (Semu DNK 234/84 and Semu DNK 65/84) while the lowest number was (0.88) between genotype (Pactol and Serw 6), (Semu DNK 234/84 and Serw 6), (Serw 6 and Semu DNK 65/84) and (Serw 6 and Agamax) (Additional file 1: Table S2). Dice's similarity index classified the canola genotypes into two main clusters. One included only Serw 6 genotype, while the second cluster, which contained 12 genotypes, was subdivided into two subclusters. The first subcluster comprised the Trapper genotype, whereas the second subcluster contained the other 11 genotypes which also were divided into two subclusters (Additional file 1: Fig. S2). From the dendrogram based on SSR analysis, the genotypes Pactol, SemuDNK 234/84 and SemuDNK 65/84 were in the same cluster.

3.8 Cluster analysis based on SSR and SRAP combined data

After the data obtained by SRAP and SSR have been analyzed individually, the binary data amplified by all the primers were combined and analyzed to evaluate the genetic relationship and similarity among the 13 canola genotypes. The genetic similarity measured utilizing the SRAP and SSR combined data analysis ranged from 0.70 between AD 201 genotype and Topas genotype while the lowest number was 0.36 between Serw 6 genotype and Serw 4 genotype (Table 14). The cluster analysis dendrogram constructed based on the total number of alleles generated by SRAP and SSR primers divided into 2 main clusters; the first cluster included Serw 6 genotype alone, while the second cluster has divided into 2 subclusters;

Table 14 The genetics similarity based on combined data

	Serw 4	HE you 46	Pactol	RG 4605	Semu DNK 234/84	HE you 56	Serw 6	Wang you 25	Semu DNK 65/84	AD 201	Topas	Agamax	Trapper
Serw 4	1.00												
HE you 46	0.53	1.00											
Pactol	0.52	0.49	1.00										
RG 4605	0.51	0.48	0.66	1.00									
Semu DNK 234/84	0.54	0.55	0.63	0.67	1.00								
HE you 56	0.48	0.43	0.63	0.59	0.55	1.00							
Serw 6	0.36	0.37	0.43	0.44	0.41	0.45	1.00						
Wang you 25	0.56	0.49	0.61	0.60	0.61	0.64	0.40	1.00					
Semu DNK 65/84	0.51	0.48	0.64	0.61	0.58	0.63	0.45	0.68	1.00				
AD 201	0.50	0.49	0.62	0.62	0.60	0.59	0.47	0.63	0.67	1.00			
Topas	0.50	0.45	0.57	0.59	0.55	0.60	0.42	0.65	0.64	0.70	1.00		
Agamax	0.48	0.44	0.55	0.55	0.53	0.53	0.41	0.59	0.59	0.62	0.57	1.00	
Trapper	0.48	0.47	0.54	0.54	0.53	0.51	0.38	0.61	0.60	0.60	0.58	0.58	1.00

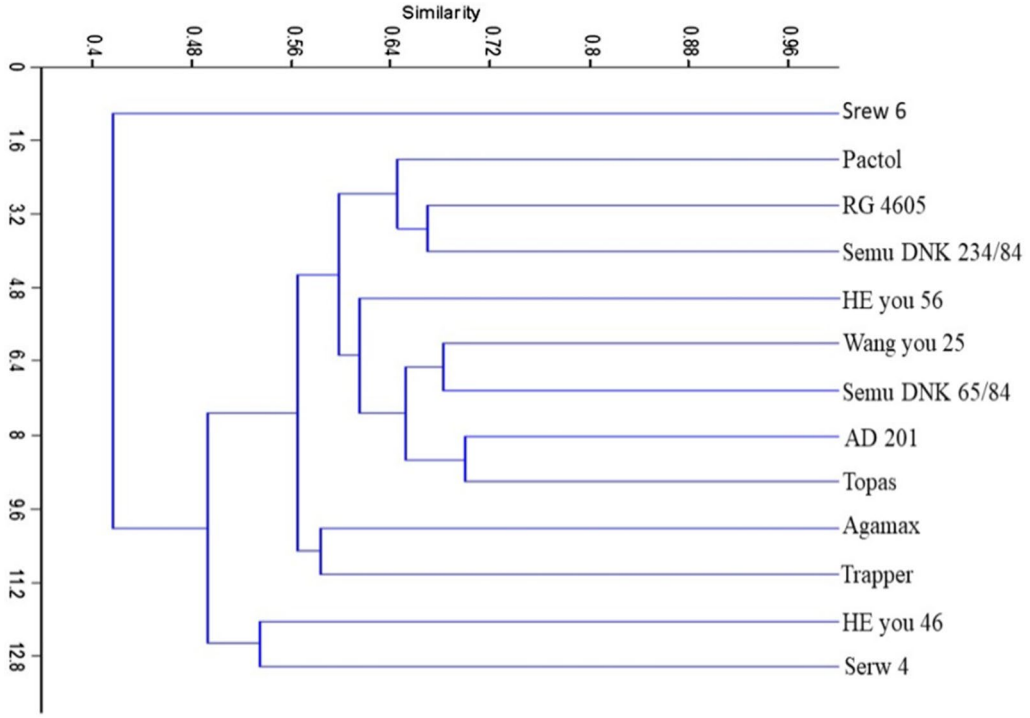

Fig. 3 Cluster analysis based on Dice similarity index derived from the combined data of the two markers

the first included Serw 4 genotypes and HE you 46 genotype, while the second has divided into 2 subclusters (Fig. 3).

4 Discussions

Different factors affect the growth parameters and seed yield in plants including genotypes, location, season, planting date, soil nutrients and growing conditions [40, 41]. Zhang et al. [41] displayed that the seed yield was significantly different among different canola genotypes. In addition, El Habbasha and Abd El salam [42] pointed out there were important differences among canola genotypes in the seed yield. The results shown in Tables 6 and 7 displayed that there were significant differences among tested genotypes in all of the studied traits under Egyptian conditions. The results showed that some canola genotypes were surpassed in their plant height, plant dry weight/plant, leaves/plant, fresh weight/plant and branches/plant such as Trapper, Agamax and Topas. This was certainly due to the genetic buildup of the genotypes under study. Different responses of other Chinese genotypes under Egyptian conditions were reported by many authors [34]. The differences among genotypes also may be attributed to their genetic constitution [10, 43, 44]. Similar results were observed by Mekki and El-Kholy [45], Singh et al. [46], Sana et al., [47], Zhang et al., [41] and Mekki [44].

In the present investigation, 36 SRAP combinations and 20 SSR markers were utilized to investigate the genetic diversity among 13 genotypes of canola. SRAP analysis resulted in a total of 742 bands detected among the canola genotypes. Only 659 of them were polymorphic bands (88.8%) (Table 4). The SRAP markers targeting ORFs as functional sequences of the canola genome displayed sufficient polymorphism. All the SSR markers were amplified generating 44 bands. Out of 20 markers, only 12 markers were polymorphic. These findings confirm the effectiveness of SSR markers when utilized to assess genetic diversity. SSR markers are the most recommended markers to evaluate the genetic variation among different canola genotypes. Ahmad et al. [48] reported that the SRAP and SSR markers were highly beneficial and revealed considerable genetic difference among 77 canola genotypes. The study of the genetic diversity between different species and genotypes of plants is very important for crop preservation and improvement [11]. Recently, several researches on genetic diversity in canola have been performed throughout the world utilizing several molecular markers [48, 49]. However, the ultimate aim of the evaluation of the genetic diversity in available

canola genotypes is to effectively use them in the breeding programs. The results of the present study could be used in breeding programs to obtain canola hybrids between the tested genotypes and the local genotypes or to cultivate the Trapper, Agamax and Topas genotypes under Egyptian conditions.

5 Conclusion

The all canola genotypes used were grown successfully under Egyptian conditions, but some genotypes surpassed in yield production such as Trapper, Agamax and Topas genotypes.

Abbreviations

SRAP	Sequence-related amplified polymorphism
SSR	Simple-sequence repeats
Brassica napus	*B. napus* L.
cm	Centimeter
g	Gram
Brassica rapa	*B. rapa*
Brassica oleracea	*B. oleracea*
RFLP	Restriction fragment length polymorphism
AFLP	Amplified fragment length polymorphism
RAPD	Random amplified polymorphic DNA
SACR	Sequence characterized amplified regions
SNP	Single nucleotide polymorphisms
ORFs	Open reading frames
NRC	National research center
DMF	*N*,*N*-Dimethylformamide
CTAB	Cetyltrimethylammonium bromide
PCR	Polymerase chain reaction
LSD	Least significant difference
PIC	Polymorphism information content
MI	Marker index
RP	Resolving power

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s43088-023-00388-3.

Additional file 1. Supplementary file.

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Author contributions
E.S.F., S.A. and N.I.E. contributed to conceptualization; S.S., E.S.F., S.A. and N.I.E. contributed to methodology; S.S., E.S.F., S.A. and N.I.E. contributed to investigation; S.S., E.S.F., S.A. and N.I.E. contributed to formal analysis; E.S.F., S.A. and N.I.E. contributed to resources; E. S.F., S. A. and N.I.E. contributed to validation; S.S., E.S.F., S.A. and N.I.E. contributed to visualization; S.S., E.S.F., S.A. and N.I.E contributed to writing-original draft preparation. All authors have read and agreed to the published the manuscript.

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Declarations

Ethics approval and consent to participate
No any animal or human were used during this experimental study.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no conflict of interest.

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