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Original article

Association of saponin concentration, molecular markers, and biochemical factors with enhancing resistance to alfalfa seedling damping-off

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ABSTRACT

Fifteen alfalfa populations were tested for resistance to the seedling damping-off disease sourced by *Rhizoctonia solani*, *Fusarium solani*, and *Macrophomina phaseolina*. In a laboratory experiment, saponin treatment significantly diminished the mycelial growth of the causal fungi of alfalfa damping-off disease. Roots of the fifteen alfalfa populations varied in saponin and lignin content. Selection for the considerably resistant plants leads to the best growth performance, desirable yield, and high nutritive values such as crude protein (CP), crude fier (CF), nitrogen free extract (NFE), ash, and ether extract (EE) contents. For the PCR reaction, 10 SSR pairs of the JESPR series primers and the cDNA-SCoT technique with seven primers were used. SSR and SCoT revealed some unique markers that could be linked to resistance to damping-off disease in alfalfa that appeared in the considerably resistant alfalfa population (the promised pop.). SSR and SCoT markers can be an excellent molecular method for judging genetic diversity and germplasm classification in tetraploid alfalfa. We recommend breeding for saponin concentration in the alfalfa plant may affect resistance to some diseases like root rot and damping-off because saponin might improve plant growth, yield, and nutritional values.

1. Introduction

Alfalfa (*Medicago sativa* L.) is the most common forage crop (Small, 1996) and is commonly used as animal feed in some forms, including green forage, dry hay, and leafy protein concentrates (Stochmal et al., 2001) to enrich animal diets with vitamins, carbohydrates, β-carotene, digestive enzymes, and high-quality proteins (Hatfield, 1992; Goławska et al., 2010). The local alfalfa populations (e.g., Ismailia 1 and Siwa) expressed the highest yield supremacy and agronomic characteristics compared to the imported populations. They are sorted as highly resistant to seedling damping-off, as well biochemical markers have been discovered to correlate with resistance levels (Abd El-Naby et al., 2014). Omar et al. (2016) confirmed that selection for the most prominent and healthiest root system of a plant could be considered by plant breeders looking for a high yielding capacity of the alfalfa plant.

Crop productivity is hindered by several stressors, including salinity (ElSayed et al., 2020; Seleiman et al., 2020; Taha et al., 2020; Azzam et al., 2021; Semida et al., 2016; Rady et al., 2021b), limited water; drought (Alharby et al., 2021a; Desoky et al., 2021; Rady et al., 2021a, Semida et al., 2021, Abdelsalam et al., 2021), the heavy metal cadmium (Semida et al., 2018; Alharby et al., 2021b), calcareous state (Awad et al., 2021; Bamagoos et al., 2021), and nutrient deficiency (Rehman et al., 2018). Besides, it is also influenced by diseases sourced by various

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pathogens, *viz* bacteria, fungi, viruses, or pests, which affect yields, yield quality, and safety. While pesticides are now associated with controlling pests and pathogens, the growing difficulties of fungal resistance to fungicide emerge to present potential risks to agriculture (Fisher et al., 2018). Saponins, including a diversified family of steroidal glycoalkaloids, steroids, or triterpenoids, originate widely in plant species (Podolak et al., 2010; Moses et al., 2014). They mainly cover part of the antimicrobial defense systems in plants (Omar, 2019, Chamkhi et al., 2022). The activity of the saponin mechanism relies on its capacity to form complexes with sterols located in the microorganism membranes, causing membrane disorder (Sreij et al., 2019; Majak et al., 1980). Saponins (e.g., zanhic acid, soyasapogenol glycosides, and medicagenic acid) are found in relatively high concentrations ranging from 0.8 to 2.0% according to the different varieties of alfalfa (Pecetti et al., 2006; Stuteville and Skinner, 1987). Resistance to downy mildew disease in alfalfa has been notably altered during selection for the high saponin content forage (Bornet and Branchard, 2001).

Breeding tactics to regularly develop new cultivars contain intermitting numerous individually selected larger plants into a crossbreeding block. One way to categorize maximally diverse parental genotypes is to assess genetic variation across molecular markers, which deliver appreciated info in the breeding of crops, primarily in determining genetic variation and relationships among crop species (Khalifa et al., 2006; Azzam et al., 2012; Azzam et al., 2015; Azzam and Khalifa, 2016; Bosily et al., 2018). Remarkably, the polymerase chain reaction (PCR) is employed to detect the amplification fragment length polymorphism (AFLP), random amplified polymorphic DNA (RAPD), simple sequence repeats (SSR), and inter-simple sequence repeats (ISSR). ISSR marker detection is performed *via* repeaters' anchored primers, amplified between SSRs, and employed for genetic diversity estimation among plant species. Due to its distinguished polymorphism and repeatability, in addition to its quite informative, ISSR is also appropriate for estimating genetic variation in diverse field crops (Wang et al., 1994; Azzam and Abo- Doma, 2007; Azzam et al., 2007; Falahati-Anbaran et al., 2007; Etminan et al., 2016; Azzam et al., 2019; Abouseada et al., 2020). SSR markers are codominant, multiallelic, exceedingly reproducible, and act with low-quality DNA (Azzam et al., 2010; Gupta and Varshney, 2000). Numerous SSR markers have been evolved and are more widely employed in plants for population genetics, gene mapping, gene diversity estimation, and marker-supported selection (Falahati-Anbaran et al., 2007; Al-Taweel et al., 2021). Recently, numerous innovative alternative and talented marker procedures have been established. SCoT (start codon targeted polymorphisms) depends on the short-kept section in plant genes surrounding the ATG translation start or initiation codon and is a reproducible marker. SCoT markers have been efficiently employed to estimate genetic structure, diversity, and classify accessions and quantitative trait loci (QTL) mapping and DNA fingerprinting in numerous species (Etminan et al., 2016; Collard and Mackill, 2009; Abd El-Naby et al., 2013).

Our objectives are to a) test alfalfa populations for damping-off disease, b) identify best resistant alfalfa populations by estimating their fresh herbage, potential yield of dry matter, and nutritional value, c) describe the relationship between alfalfa resistant root lignin and saponin concentrations and resistant to three soil-borne fungi, d) describe the use of SSR and SCoT markers to examine the genetic variation among alfalfa populations and to identify alfalfa DNA markers linked to alfalfa seedling damping-off disease resistance, to clearly define the genetic basis of resistance to these stressors and accelerate breeding programs. This procedure could be utilized to achieve the best perception of biodiversity conservation and genetic resources in designing cultivated alfalfa breeding programs.

2. Materials and methods

2.1. Plant sources

Fifteen alfalfa populations were tested against three fungi that cause the seedling disease "damping-off," *viz Fusarium solani, Rhizoctonia solani*, and *Macrophomina phaseolina*. The genetic materials comprised eleven Egyptian landraces in addition to a promising pop. more suitable for saline conditions of North Sinai soils (Abd El-Naby et al., 2013). One local cultivar and two exotic populations were obtained from the Forage Crop Department (FCRD), FCRI, ARC, Giza, Egypt. Table 1 presents the origin of these populations. The populations were investigated at Agricultural Research Station, ARC, during 2017 and 2018.

2.2. Total saponin and lignin

Total saponin and lignin (%) of root dry matter were analyzed in the Regional Food and Feed Center Lab, ARC, by High-Performance Liquid Chromatography (HPLC) described by Oleszek et al. (1990).

2.3. Saponin laboratory experiment

Different concentrations of saponin were added to the PDA (potato dextrose agar) medium to obtained final concentrations of 1, 3, and 5 g/L. Then the media were autoclaved to study the influence of saponin on mycelia growth of *F. solani,* and *R. solani, M. phaseolina*. The forenamed media were poured into Petri dishes (9 cm diam.). Three replicates were used for each concentration. Plates containing only PDA medium were used as control treatment. All plates were inoculated with discs 6-days-old-cultures of *R. solani, M. phaseolina,* or *F. solani* individually and then incubated at 25 °C. When the control of each fungus covered the dishes, the diameter of the fungal growth was recorded, and the reduction percentage was computed with the following formula:

$$R\% = (C - T)/C \times 100$$

where R = fungal growth reduction percentage, C = fungal growth in the control treatment, and T = fungal growth in the applied treatment.

2.4. Pathogenic studies

The fungi *Fusarium solani, Rhizoctonia solani*, and *Macrophomina phaseolina* were previously isolated from the rotting alfalfa plant roots, collected from the Research Station, Agricultural Research Center (ARC). The fungi were purified and identified (Barnett, 1960). Besides, the fungi pathogenicity was proved in Legume and Forage Disease Research Department, Plant Pathology Research Institute, ARC.

2.5. Fungal inoculum preparation

Bottles containing 3 cornmeal l: 1 sand medium (w/w) were prepared for 30 min of sterilization at 121 °C. They were then individually inoculated with 5 mm diameter discs from 7-d-old-cultures of *Fusarium solani, Rhizoctonia solani, and Macrophomina phaseolina.* Then, the bottles were prepared for 15-d incubation at 25 °C.

2.6. Soil infestation

The sterilized medium was mixed with the inoculum of each of *Fusarium solani, Rhizoctonia solani, and Macrophomina phaseolina* at 5, 3, and 5% (w/w/w), respectively. Daily for a week, the infected soils were watered to promote the inoculum growth and distribu-

Table 1
The code and origin of tested alfalfa populations.

Code No.	Population	Origin	Code No.	Population	Origin
1	Wadi-1	New valley (local)	**9**	Siwa-2	Siwa Oasis (Local)
2	Wadi-2	New valley (local)	**10**	Siwa-3	Siwa Oasis (Local)
3	Wadi-3	New valley (local)	**11**	Promised pop.	High tolerant salt stress pop.
4	Wadi-4	New valley (local)	**12**	Nubaria	Nubaria (Local)
5	Wahat-1	Wahat Oasis (local)	**13**	Rammah	Local cultivar
6	Wahat-2	Wahat Oasis (local)	**14**	Cuf-101	Exotic-America
7	Wahat-3	Wahat Oasis (local)	**15**	Sea-reiver	Exotic- Australia
8	Siwa-1	Siwa Oasis (Local)			

tion. The seeds of the fifteen alfalfa populations were sown using pots with the infected soils (Table 1), 30 seeds pot^{-1}, three replicates for each alfalfa population.

The experiment was examined periodically, and at 45 d after sowing, the seedlings' damping-off was recorded. The reaction of all populations tested to the infection was scored according to the following scale:

Resistant (R) = ≤25% decrease in the survival plants,
Moderate susceptible (MS) = 26–35% decrease in the survival plants,
Susceptible (S) = 36–45% decrease in the survival plants and
Highly susceptible (HS) = ≥45% decrease in the survival plants.

2.7. Agronomical characters

Selection for the considerably resistant plants (a ≤ 25% reduction in the survival plants per population) was practiced. Ninety plants were detected over three tested fungi and then were blended and transplanted in the field after 60 days from sowing. Also, the entries were positioned in a completely randomized block design with three replicate. An area of 3 m^2 (2 × 1.5) was allocated to each experimental plot with three rows and 25 cm row spacers, ten plants per row. Before transplanting and after transplant preparation, fertilizer [superphosphate (15.5% P_2O_5), urea (46 % N), potassium sulfate (48% K_2O)] were applied according to standardized alfalfa recommendation. Ten days after transplanting, the transplants were watered as the first watering, and then watering was carried out after each cut. Ten cuts were taken over 65 days from transplanting. The agronomic characteristics were examined for plant height (cm), tillers per m^2, total yields per each fresh and dry plant, and unit area (m^2) for each cut. A sample of 200 g of fresh forage was collected from each plot to dry at room temperature with indirect exposure to sunlight to estimate the dry weight of the forage. The dry weight was recorded when the samples reached a constant weight for three weights on three days. The dry matter (% DM) production was computed for each treatment.

2.8. Nutritive determinations

Utilizing the content of N, crude protein (CP) content was computed (CP = N × 6.25) (Bozkurt and Kaya, 2010). The total content of ash was determined (James, 1995). The procedures depicted in AOAC (2005) were applied to determine each ether extract (EE), crude fiber (CF %), and crude ash. Nitrogen free extract (NEF) concentrations were determined applying the following equation: NFE = 100 − (CP% + CF% + EE% + Ash%) (AOAC, 2005).

2.9. Estimation of biochemical content in alfalfa roots

100 g root dry weight samples per population were finely powdered to determine saponin and lignin percentages. The experiments were carried out in Regional Center for Food and Feed (ARC), Giza.

Total saponin was estimated by High-Performance Liquid Chromatography (HPLC) (Oleszek et al., 1990). Also, Lignin acid detergent fiber (ADL) content was determined by Near-Infrared Spectrophotometry (NIRS) (Dale et al., 2013).

2.10. Statistical analysis

Data were statistically analyzed utilizing the SAS (SAS Institute, Inc., Cary, NC), as well as ANOVA and Duncan's Multiple Range tests (Duncan, 1955).

2.11. Molecular studies

The following protocols were carried out at the Cell Research Department, FCRI, ARC to estimate the genetic distance among the fifteen alfalfa populations, look for genotype-specific markers, and find molecular genetic markers linked to resistance and susceptibility to seedling damping-off disease.

2.12. DNA preparation and SSR loci amplification

Leaves from the 15 alfalfa populations were employed to isolate DNA, applying the Doyle and Doyle (1987) CTAB method with a modification (Khaled and Esh, 2008). The purity of the DNA was estimated from the A260/A280 ratio (more than 1.79 on an average for all populations). All the samples were found to be RNA-free with no signs of any degradation during preparation. The DNA yield was ranged from 500 to 800 ng/mg leaf fresh mass for all individual samples. Twenty SSR pairs of the JESPR series primer (Reddy et al., 2001) were screened using the samples of DNA of each alfalfa population and based on this preliminary data. Ten SSR primers were selected (Table 2).

PCR amplification of genomic DNA was implemented in a reaction volume of 25 μL utilizing an ABI 2700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA), which was contained 2.5 μL 10x PCR buffer [Tris–HCl (0.1 M), pH 8.8 at 25 °C, KCl (0.5 M), 0.8 % (v/v) Nonidet] + ½ μL from dNTPs (0.01 M) + 1 U of Taq DNA polymerase (Sangon, Shanghai, China) + ½ μL from of each primer (0.01 mM) + 0.002 mL from $MgCl_2$ (0.025 M) + 50 ng of template DNA. The protocol of PCR involved initial denaturation of DNA at 94 °C for 5 min, followed by 35 denaturation cycles at 94 °C for 30 sec, then annealing at 55 °C for 30 sec and extension at 72 °C for 1 min. It ended with a final extension of 72 °C for 4 min, then held at 4 °C (infinite). The products that resulted from the PCR protocol were stored at − 20 °C.

The products of PCR were moved to electrophoresis (90 V) in agarose gel (2%) containing ½ μg ethidium bromide mL^{-1} for about 2 h using ½ × TBE buffer with a DNA ladder. Fragments were detected on a UV transelumentor and imaged utilizing a gel documentation system (Alpha Ease FC, Alphimager™ 2200, USA).

Table 2
The 10 SSR forward (F) and reverse (R) primers of the JESPR series.

Primer	Oligo	SEQ	Repeat type	MER	GC%	Tm (°C)
1	JESPR247-F	5′-GCTTCTTCCATTTTATTCAAG-3′	$(CT)_{15}$	21	33.33	53.5
	JESPR247-R	5′-CAGCGGCAACCAAAAAG-3′		17	52.94	52.4
2	JESPR284-F	5′-CAAGATCCATCTGCTGATTAG-3′	$(CA)_{25}$	21	42.86	57.4
	JESPR284-R	5′-CTATATACAAGTATAAAGTATTGG-3′	$(TA)_5$	24	25.0	55.0
3	JESPR291-F	5′-CATTCCCCACTTTGCTCTTAC-3′	$(CTT)_8$	21	47.62	59.4
	JESPR291-R	5′-CATGTTTCTTTGCCCATC-3′		18	44.44	51.6
4	JESPR292-F	5′-GCTTGCAATCTCCTACACC-3′	$(CTT)_7$	19	52.63	57.3
	JESPR292-R	5′-GAATATGTTTCATAGAATGGC-3′		21	33.33	53.5
5	JESPR293-F	5′-CGAGATTTTAAGATTGTGC-3′	$(GAA)_7$	19	36.84	50.9
	JESPR293-R	5′-TGATGGCAAAAGCACC-3′		16	50.0	48.2
6	JESPR295-F	5′-GCCTCGTTTAAGCCCATAAAC-3′	$(CTT)_7$	21	47.62	59.4
	JESPR295-R	5′-GAGGGCCATAGTCACCGG-3′		18	66.67	60.7
7	JESPR300-F	5′-CGCATCACAAACCAAACAC-3′	$(CTT)_5$	19	47.37	55.2
	JESPR300-R	5′-CGGAAAATGATGATGATGAAGAAG-3′	$(CAT)_6$	24	37.5	60.1
8	JESPR301-F	5′-TGAGTTCCGAATTCCTTGG-3′	$(CAT)_8$	19	47.37	55.2
	JESPR301-R	5′-CGGGCTAAGTGTTTTTCG-3′		18	50.0	53.9
9	JESPR302-F	5′-CACTCCTAGCTTCTTGGCATC-3′	$(GAT)_5$	21	52.38	61.3
	JESPR302-R	5′-CTGCGATCTTGGCACAG-3′		17	58.82	54.8
10	JESPR304-F	5′-GAAATGCATTCCCTCAAAAGC-3′	$(GAT)_8$	21	42.86	57.4
	JESPR304-R	5′-AGACTCTATCGAATGACCCTG-3′		21	47.62	59.4

Table 3
Details of 7 SCoT primers sequences used in PCR reaction.

Primer	Oligo	SEQ Primers sequence (5′–3′)	GC%	Tm (°C)
1	SCoT1	5′- CAACA**ATG**GCTACCACCA-3′	50.0	53.9
2	SCoT2	5′-CAACA**ATG**GCTACCACCC-3′	55.56	56.1
3	SCoT3	5′-CAACA**ATG**GCTACCACCG-3′	55.56	56.1
4	SCoT4	5′-CAACA**ATG**GCTACCACCT-3′	50.0	53.9
5	SCoT5	5′-CAACA**ATG**GCTACCACCA-3′	50.0	53.9
6	SCoT6	5′-CAACA**ATG**GCTACCACGC-3′	55.56	56.1
7	SCoT7	5′-CAACA**ATG**GCTACCACGG-3′	55.56	56.1

2.13. cDNA SCoT PCR reaction and amplification conditions

The cDNA-SCoT technique was used as described in (Al-Taweel et al., 2019). The RNA was extracted from fifteen alfalfa populations according to the Trizol method (Luo et al., 2014). The RNA synthesized cDNA by adding 1 µL of oligo dT to RNA and incubating at 66 °C for 5 min. After thawing on ice for two minutes, reverse transcriptase 1 µL 5 × buffers, two µl of dNTPase, and 1 µL of reverse transcriptase enzyme were added. Every alfalfa sample was incubated for 1 cycle in PCR at 42C for 1 h followed by another termination cycle at 70 °C for 5 min. cDNA concentration was measured using Fluorometer, and 100 ng of cDNA was used to conduct the reaction for all alfalfa samples. The cDNA-SCoT technique was applied to compare the 15 alfalfa populations and find molecular markers linked to alfalfa seedling damping-off disease due to different gene expressions. Seven primers (cDNA- SCoTOligo primer, macro gene Company) were used (Table 3). All the reaction mixture components (25 µL) were gathered to amplify and evolve the SCoT markers. PCR reaction was implemented on ABI 2700 Thermal Cycler (Applied Biosystems, Foster City, CA, USA). Initial denaturation set out at 95 °C for 4 min, followed by 40 cycles at 95 °C for 51 °C for 1 min, 72 °C for 1 min, and a final extension at 75 °C for 5 min. The amplification products were separated on agarose gel (1.3%), which contained ethidium bromide against a 100 bp DNA ladder. Fragments were detected on a UV transelumentor and photographed with a gel documentation system (Alpha Ease FC, Alphimager™ 2200, USA).

Table 4
Influence of different saponin concentrations on mycelia growth % of *M. phaseolina*, *R. solani*, and *F. solani*.

Fungi	Concentration (g/L)	Mycelia growth (cm)	Reduction (%)
M. phaseolina	**0.0 (Control)**	9.00a	–
	1.0	7.37b	18.55
	3.0	5.73c	36.33
	5.0	4.87d	45.89
Mean		**6.74**	**33.59**
L.S. D. (0.05)		**0.5844**	–
R. solani	**0.0 (Control)**	9.00a	–
	1.0	7.33b	15.89
	3.0	6.67c	31.89
	5.0	6.47c	33.67
Mean		7.37	**27.15**
L.S. D. (0.05)		**0.6095**	
F. solani	**0.0 (Control)**	9.00a	–
	1.0	7.40b	18.55
	3.0	6.13c	25.89
	5.0	5.97c	28.11
Mean		7.13	**24.18**
L.S. D. (0.05)		**0.5338**	**–**

Different letters behind each of two consecutive mean values within each column in the Table indicate a significant difference at $p \leq 0.05$.

2.14. Molecular data analysis

The banding patterns generated by SSR and SCoT primers were scored as present (1) or absent (0) for each primer using 1D software (Total Lab software v2009, Nonlinear Dynamics, UK). The genetic similarities were computed following Dice, and the dendrogram was created using SPSS windows version 22 (Yang and Quiros, 1993).

3. Results

3.1. Saponin laboratory experiment

Data in Table 4 show a significant ($p \leq 0.05$) reduction in mycelial growth of the tested fungi due to the application of saponin. With the increase in the saponin concentration, the growth rate of the fungi subsequently decreased. The reduction ranged

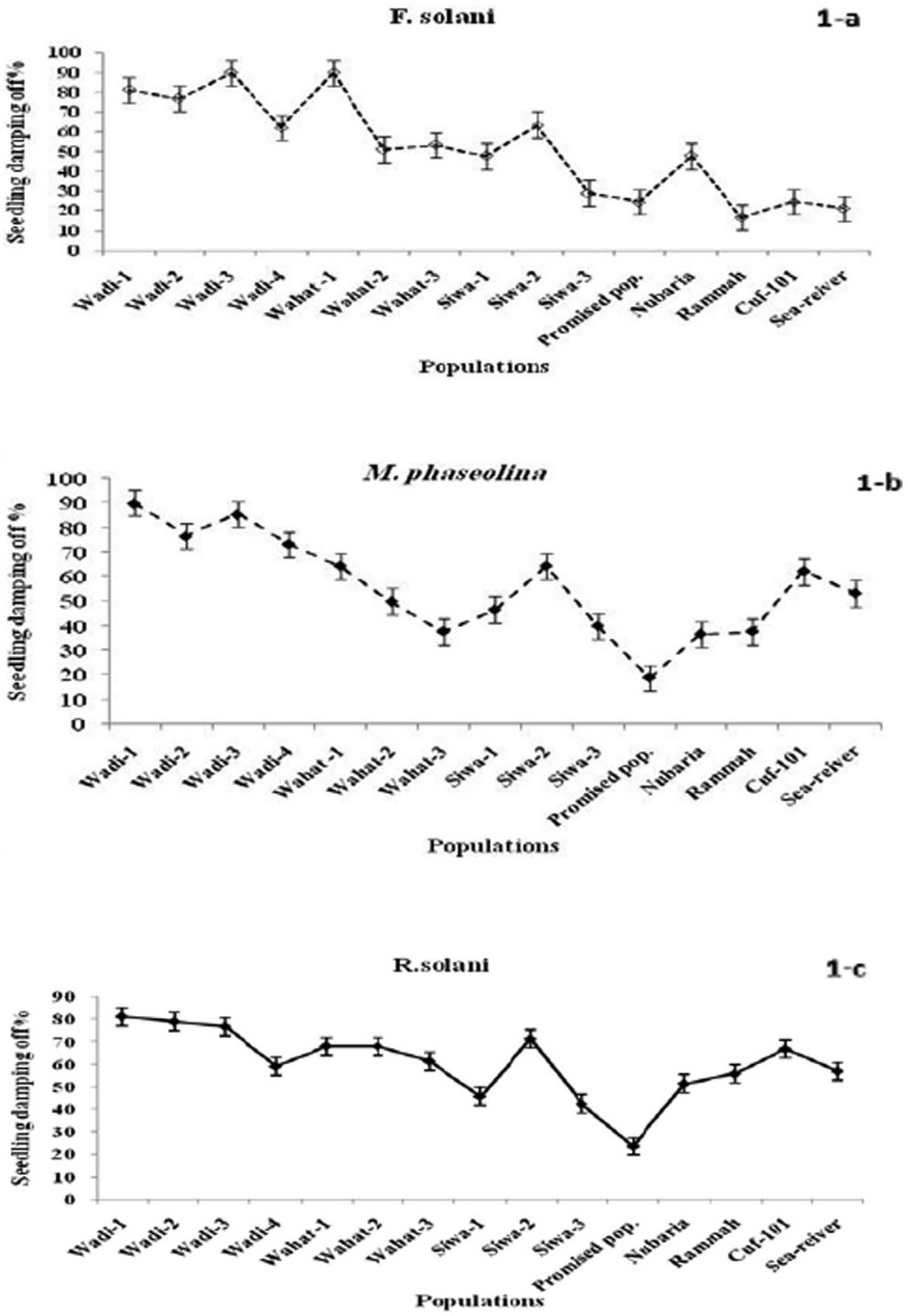

Fig. 1. The effect of 3 tested fungi on alfalfa seedling Damping-off % ±SE (Standard error), (**a**) Fusarium solani, (**b**) Macrophomina phaseolina, and (**c**) Rhizoctonia solani.

Table 5
Means of plant height (cm), number of tillers/m^2, fresh herbage weight /plant (FWg/plant), dry herbage weight/plant (DWg /plant) and fresh herbage (FYg/m^2), and dry yield (DYg/m^2) across ten cuts of fifteen alfalfa selected populations.

Code No.	Population	Plant height (cm)	Tillers/m^2	FW/plant (g)	DW/plant (g)	FY/ m^2 (g)	DY/ m^2 (g)
1	Wadi-1	75.67^{cd}	277.67^{fgh}	402.33^{f}	104.87^{g}	3621.0^{efg}	1103.8^{efg}
2	Wadi-2	71.33^{efg}	267.33^{gh}	337.50^{hi}	95.13^{gh}	3270.8^{feh}	1002.9^{fgh}
3	Wadi-3	72.67^{def}	255.33^{i}	342.17^{hi}	96.63^{gh}	3179.5^{gh}	903.0^{fgh}
4	Wadi-4	70.67^{fg}	266.67^{h}	423.83^{f}	114.87^{def}	3814.5^{de}	1033.8^{defg}
5	Wahat-1	79.00^{bc}	291.00^{bcd}	429.33^{ef}	123.33^{de}	3964.0^{de}	1240.2^{cd}
6	Wahat-2	80.33^{a}	289.00^{cde}	466.17^{e}	126.30^{d}	4195.5^{d}	1266.6^{cd}
7	Wahat-3	80.33^{a}	302.00^{ab}	643.00^{b}	178.93^{b}	5145.0^{ab}	1410.0^{ab}
8	Siwa-1	70.33^{fg}	267.00^{gh}	347.33^{gh}	95.80^{gh}	3126.0^{hi}	1012.2 ^{efg}
9	Siwa-2	66.67^{h}	226.00^{k}	298.83^{j}	84.67^{i}	2689.5^{j}	862.2^{i}
10	Siwa-3	77.00^{bcd}	285.00^{def}	529.00^{d}	145.43^{c}	4761.0^{c}	1308.8^{bc}
11	Promising pop.	79.67^{b}	308.33^{a}	671.67^{a}	181.53^{a}	5345.0^{a}	1451.3^{a}
12	Nubaria	74.33^{de}	240.00^{j}	338.50^{hi}	94.73^{gh}	3313.2^{fgh}	1023.6^{efg}
13	Rammah	72.17^{efg}	267.00^{gh}	432.50^{ef}	118.23^{def}	3892.5^{de}	1264.0^{cd}
14	Cuf-101	73.33^{de}	299.33^{abc}	571.67^{c}	156.70^{c}	5087.0^{bc}	1360.5^{bc}
15	Sea-reiver	70.00^{hg}	278.00^{fgh}	394.83^{fg}	109.37^{efg}	3686.8^{ef}	1050.9^{def}
Mean		74.73	274.64	441.91	121.76	3399.41	1146.24

Different letters behind each of two consecutive mean values within each column in the Table indicate a significant difference at $p \leq 0.05$.

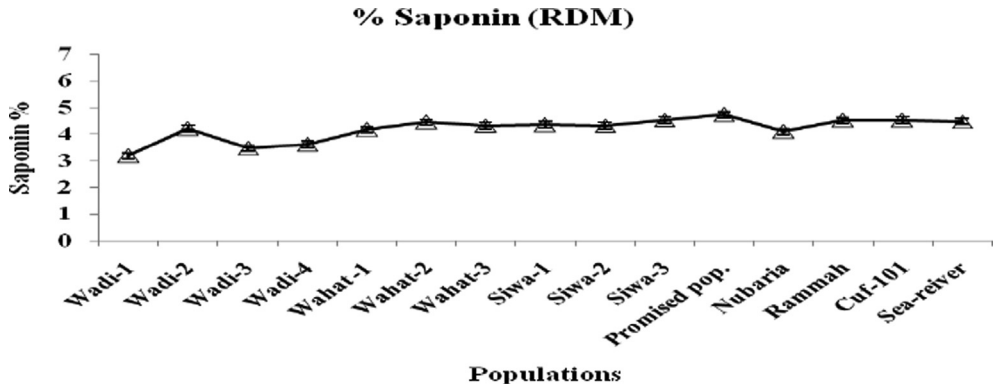

Fig. 2. The saponin content of fifteen alfalfa root dry matter samples ± SE (Standard error).

between 28.11 and 45.89%. *M. phaseolina* recorded maximum reduction followed *by R. solani* and *F. solani*, respectively.

3.2. Different alfalfa population reactions against seedling damping-off disease sourced by F. solani, R. solani, and M. phaseolina

The differences in alfalfa populations' susceptibility to infection with *F. solani, R. solani,* and *M. phaseolina* are presented in Fig. 1a. The promised pop., Rammah, Cuf-101, and Sea reiver populations were resistant to *F. solani,* agreeing with the fungal infection mentioned scale, judged by the lowest damping-off seedlings, whereas, Siwa-3 population performed moderate susceptibility. Other tested populations were highly susceptible to *F. solani*. The effect of *M. phaseolina* on the promised pop. (Fig. 1b) clarified that it was resistant, while Whahat-3, Siwa-3, Rammah, and Nubaria populations were susceptible. All of the other tested populations verified high susceptibility. All studied populations tainted high susceptibility to *R. solani* except promised pop. the resistant one, whereas Siwa-3 population was susceptible (Fig. 1c). It could be conducted that promised pop. was resistant to all tested fungi.

3.3. Agronomic traits

Data depicted in Table 5 displayed the statistical analyses of means/plant traits among ten cuts. High noticeable differences ($P \leq 0.01$) were noticed with plant height, number of tillers/m^2, fresh herbage weight (g FW/plant), dry herbage weight (g DW/-plant), and fresh herbage (FY) and dry yield (DY) across ten cuts of the fifteen tested alfalfa populations.

Plant height means varied from 66.67 cm (Siwa-2 population) to 80.33 cm (Wahat-2 and 3), with an average mean of 74.73 cm across all populations. The number of tiller/m^2 indicated broad differences among the tested populations. Its high values were observed for promised pop. and Wahat-3 local populations with 308.33 and 302.0 tillers, respectively.

CUF-101 expert pop. had a better number of tillers/m^2 with 299.33 tillers. Wahat 1, 2, and 3 performed more tillers than other populations, whereas Siwa-2 pop. had the lowest one with 226.00 tillers/m^2. The averages mean of tillers/m^2 overall tested populations recorded 274.64 tillers. Promised pop. had the best fresh and dry herbage weight/plant and per m^2 unit area with (671.67, 181.53 and 5345.0, 1451.3 g, respectively) followed by Wahat-3 population with (643.0, 178.93 and 5154.0, 1410 g, respectively) across all studied populations. In contrast, Siwa-2 pop. had the lowest values over all studied traits for fresh and dry weight /plant and per m^2 (298.83, 84.67 g, and 2689.5. 862.2 g, respectively). CUF-101 pop. recorded higher data under tested conditions than Sea-reiver, the export ones. Also, local tested populations had good agronomic performances than the exotic tested ones (Table 5).

3.4. Antifungal alfalfa saponin

The total root saponin percentage ± SE of alfalfa root dry mater per each selected population is presented in Fig. 2. Saponin ana-

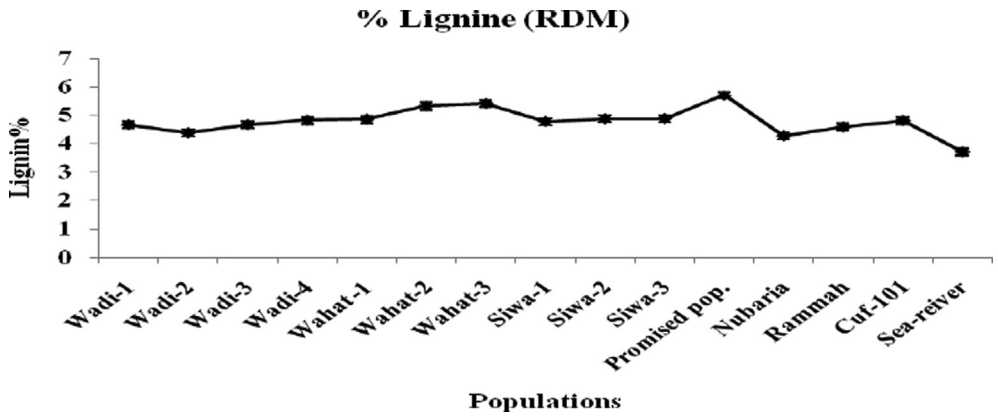

Fig. 3. The lignin content of fifteen alfalfa root dry matter samples ± SE (Standard error).

Table 6
Chemical constituents of the shoot over ten cuts of fifteen alfalfa populations. Data are based on dry shoot matter (%).

Code No.	Population	CP (%)	EE (%)	CF (%)	NFE (%)	Ash (%)
1	Wadi-1	17.5d	2.3g	20.4a	48.0k	11.8b
2	Wadi-2	18.8b	2.5f	19.6b	48.9d	10.2 k
3	Wadi-3	17.3e	2.5f	18.7de	50.1a	11.4d
4	Wadi-4	17.5d	2.6e	19.7b	49.5b	10.7 h
5	Wahat-1	17.9cd	2.7d	19.3c	48.5g	11.6c
6	Wahat-2	18.2c	2.9b	19.6b	48.2i	10.6i
7	Wahat-3	19.4a	3.1a	18.8d	48.5g	10.8 g
8	Siwa-1	18.6bc	2.7d	19.4c	48.8e	10.5j
9	Siwa-2	18.2c	2.7d	19.2c	49.0c	10.9f
10	Siwa-3	18.9b	2.8c	19.3c	48.3i	10.7 h
11	Promising pop.	19.5a	2.8c	18.5e	48.3i	10.9f
12	Nubaria	17.4d	2.5f	20.1a	47.4h	12.6a
13	Rammah	18.3c	2.6e	19.6b	48.2j	11.3e
14	Cuf-101	19.3a	2.7d	18.5e	48.7f	10.8 g
15	Sea-reiver	17.5d	2.5f	19.6b	48.8L	11.6c
Mean		18.33	19.35	2.66	48.62	11.55

Different letters behind each of two consecutive mean values within each column in the Table indicate a significant difference at $p \leq 0.05$. CP = Crude protein, CF = Crude fiber, Carbo = Carbohydrates, and EE = ether extraction.

lyzed ratios ranged from 3.18% Wadi-1 populations to 4.72% Promised pop. followed by Siwa-3, CUF-101, and Rammah populations (4.54, 4.53, and 4.52%, respectively). Studied populations recorded saponin (RDM) average mean of 4.11% overall alfalfa tested populations. High saponin percentage in some alfalfa populations encouraged plant resistance to fungal infections of *F. solani, R. solani,* and *M. phaseolina.*

3.5. Lignin root content

The lignin fungal resistance relationship is verified in Fig. 3, whereas increasing lignin percentage raised plant wall resistance to fungal infectivity. Lignin % of root dry matter content among tested populations assorted from 4.24% Nubaria population to 5.68% promised pop. followed by Wahat-2 (5.37 %) and Wahat-3 (5.29 %), respectively. Studied populations recorded lignin (RDM) average mean of 4.76% overall alfalfa tested populations. Local tested populations indicated practically comparable percentages of lignin root content.

3.6. Alfalfa shoot nutritive values

The concentrations of crude protein (CP), ether extraction (EE), crude fiber (CF), N free extract (NEF), and ash for the shoots of the selected plants per alfalfa population are depicted in Table 6. High noticeable differences ($p \leq 0.01$) were observed among the fifteen populations.

The most significant CP concentrations were detected in promised pop. followed by Wahat-3 and CUF-101 populations (19.5, 19.4, and 19.3%, respectively), whereas the lowest concentrations were presented for the Nubaria population (17.4%).

Intended for the crude fiber (CF) concentration of the alfalfa populations, Wadi-1 and Nubaria populations were the highest concentrations with the same content, 20.1%, which caused significant increases compared with promised pop. and CUF-101 populations (18.5%). The greatest concentrations of nitrogen free extract (NEF) were recorded for Wadi-3, Wadi-4, and Siwa-2 populations (50.1, 49.5, and 49%, respectively), while as Nubaria population had the lowest NEF content overall tested populations (47.4%).

The mean % of the fifteen populations scored 11.55% for the ash content, whereas the highest values recorded were 12.6% and 11.8%, respectively, for Nubaria and Wadi-1 populations. In addition, ether extraction (EE) content ranged from 3.1% Wahat-3 to 2.3% Wadi-1 with a mean of 2.66% over all the tested populations (Table 6).

3.7. Molecular studies

3.7.1. SSR analysis

Our results showed that the 10 SSR primer pairs were used to evaluate the genetic divergence among 15 alfalfa populations. All the 10 SSR primers were informative and discriminated among the alfalfa populations (Table 7 and Fig. 4), although this is the first

Table 7
The SSR primers used and their amplification results.

Primer	Primer sequence (5′→3′)	Total number of amplified bands (TNB)	The number of polymorphic bands (NPB)	Percentage of polymorphic bands (PPB) (%)	Specific markers	
					Positive unique markers (PUM)	Positive unique markers (PUM)
JESPR247-F JESPR247-R	5′-GCTTCTTCCATTTTATTCAAG-3′ 5′-CAGCGGCAACCAAAAAG-3′	10	10	100	–**1** at Promised pop. at 353.01 bp –**1** at Wadi1 at 284.93 bp –**1** at Nubaria at 244.06 bp	–
JESPR284-F JESPR284-R	5′-CAAGATCCATCTGCTGATTAG-3′ 5′-CTATATACAAGTATAAAGTATTGG-3′	10	10	100	–**1** at Nubaria at 381.19 bp – **1** at Wahat-1 at 358.78 and 45.90 bp – **1** at Sea-reiver at 291.47 bp – **1** at Wadi-3 at 57.38 bp	–
JESPR291-F JESPR291-R	5′-CATTCCCCACTTTGCTCTTAC-3′ 5′-CATGTTTCTTTGCCCATC-3′	11	11	100	–**1** at Siwa-2 at 358.78 bp – **1** at Nubaria at 57.14 bp	–
JESPR292-F JESPR292-R	5′-GCTTGCAATCTCCTACACC-3′ 5′-GAATATGTTTCATAGAATGGC-3′	10	10	100	–**1** at Wahat-3 at 53.13 bp –**1** at Siwa-2 at 43.75 bp	–
JESPR293-F JESPR293-R	5′-CGAGATTTTAAGATTGTGC-3′ 5′-TGATGGCAAAAGCACC-3′	5	5	100	–**1** at Siwa-3 at 372.42 bp	–
JESPR295-F JESPR295-R	5′-GCCTCGTTTAAGCCCATAAAC-3′ 5′-GAGGGCCATAGTCACCGG-3′	10	10	100	–**1** at Wadi1 at 378.52 bp –**1** at Wahat-2 at 340.14 –**1** at Promised pop. At 305.58 bp	–
JESPR300-F JESPR300-R	5′-CGCATCACAAACCAAACAC-3′ 5′-CGGAAAATGATGATGATGAAGAAG-3′	11	11	100	–**1** at Wahat-2 at 365.27 bp –**2** at Wadi-4 at 276.58 and 40.91 bp –**1** at Rammah at 239.12 bp –**1** at Wadi-2 at 54.54 bp –**1** at Wadi1 at 13.64 bp	–
JESPR301-F JESPR301-R	5′-TGAGTTCCGAATTCCTTGG-3′ 5′-CGGGCTAAGTGTTTTTCG-3′	11	11	100	–**1** at Rammah at 378.96 –**1** at Wahat-1 at 294.37 bp –**1** at Promised pop. At 58.33 bp	–
JESPR302-F JESPR302-R	5′-CACTCCTAGCTTCTTGGCATC-3′ 5′-CTGCGATCTTGGCACAG-3′	10	10	100	–**2** at Wadi-1 at 470.34 and 26.67 bp	–
JESPR304-F JESPR304-R	5′-GAAATGCATTCCCTCAAAAGC-3′ 5′-AGACTCTATCGAATGACCCTG-3′	10	10	100	–**1** at Promised pop. At 386.66 bp –**1** at Wahat-3 at 283.69 bp –**1** at Rammah at 240.69 bp – **1** at Wadi1 at 403.46 bp	–

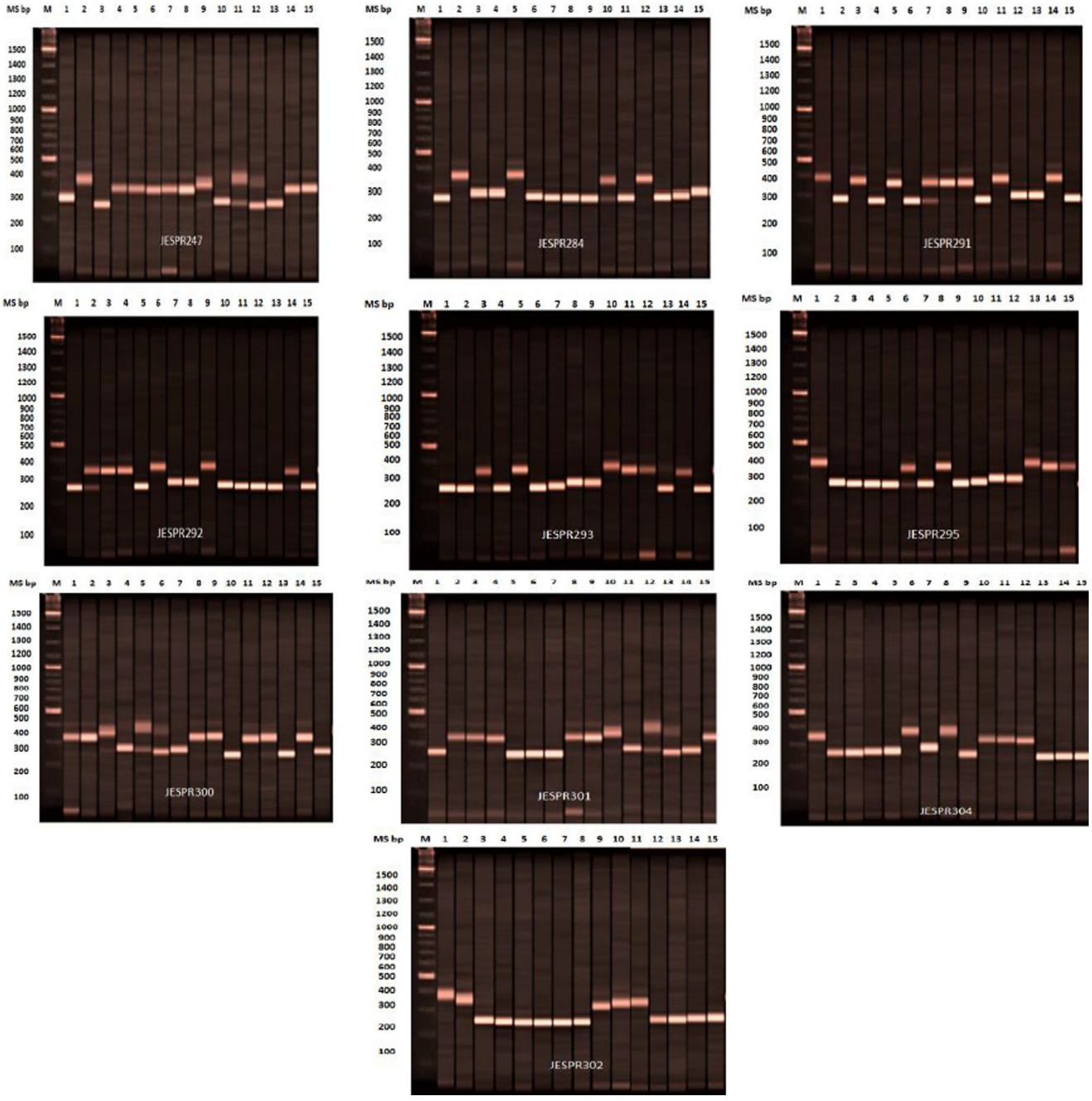

Fig. 4. Simple sequence repeat variation of fifteen Alfalfa plants using 10 SSR pair primers. The letter 'M' denotes the molecular marker, Whereas 1 = Wadi-1, 2 = Wadi-2, 3 = Wadi-3, 4 = Wadi-4, 5 = Wahat-1, 6 = Wahat-2, 7 = Wahat-3, 8 = Siwa-1, 9 = Siwa-2, 10 = Siwa-3, 11 = Promised pop., 12 = Nubaria, 13 = Rammah, 14 = Cuf-101 and 15 = Sea-reiver.

time to use this kind of primers with alfalfa. Ninety-eight alleles were detected across all polymorphic SSR primers, with several alleles per SSR locus, with a range of 5–11. Our results confirmed that the significant loci recorded were highly polymorphic, with an average of 9.8 alleles per locus.

For germplasm differentiation, four loci possess distinctive positive polymorphisms, which distinguished the promising pop. among all populations, which could be linked to the resistance of alfalfa damping-off disease. Also, 6, 1, 1, and 1 locus had positive, unique polymorphisms that separated Wadi-1, Wadi-2, Wadi-3, and Wadi-4, respectively, from the remaining populations (Table 7). Two loci in each What's population had positive, unique markers, separating them from the remaining populations. Moreover, 2 and 1 loci had positive, unique polymorphisms that distinguished Siwa-2 and Siwa-3, respectively, from the other populations. Three loci also were positive, unique polymorphisms that distinguished Rammah from the other populations, while only one locus for See-river. On the other hand, Siwa-1 and Cuf-101 may need additional molecular markers to characterize them from each other and the other populations (Table 7 and Fig. 4).

The Dice coefficient genetic similarity and UPGMA algorithm computed the 15 alfalfa populations relying on the 10 SSR markers. The genetic uniformity among alfalfa populations varied from 0.42 to 0.92. This finding indicates a high genetic similarity level among populations. Our results suggest that these populations possess a high genetic overlap because of the gene flow overseed and pollen

Table 8
Dice coefficient genetic similarity matrix among 15 alfalfa populations based on the 10 SSR markers.

Alfalfa Pop.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.77													
3	0.66	0.87												
4	0.77	0.71	0.62											
5	0.45	0.44	0.42	0.46										
6	0.49	0.52	0.54	0.46	0.75									
7	0.49	0.50	0.50	0.46	0.69	0.92								
8	0.51	0.53	0.58	0.48	0.61	0.79	0.82							
9	0.65	0.60	0.67	0.59	0.56	0.70	0.69	0.76						
10	0.65	0.65	0.71	0.61	0.52	0.70	0.69	0.73	0.90					
11	0.61	0.57	0.68	0.56	0.59	0.64	0.61	0.63	0.77	0.75				
12	0.52	0.56	0.53	0.47	0.68	0.71	0.70	0.66	0.67	0.65	0.62			
13	0.50	0.56	0.59	0.49	0.43	0.56	0.54	0.51	0.61	0.59	0.62	0.61		
14	0.46	0.59	0.60	0.50	0.44	0.59	0.57	0.54	0.67	0.71	0.57	0.64	0.80	
15	0.45	0.47	0.44	0.52	0.49	0.56	0.55	0.55	0.61	0.61	0.49	0.62	0.70	0.70

Whereas, 1 = Wadi-1, 2 = Wadi- 2, 3 = Wadi-3, 4 = Wadi-4, 5 = Wahat-1, 6 = Wahat-2, 7 = Wahat-3, 8 = Siwa-1, 9 = Siwa-2, 10 = Siwa-3, 11 = Promising pop., 12 = Nubaria, 13 = Rammah, 14 = Cuf-101 and 15 = Sea-reiver.

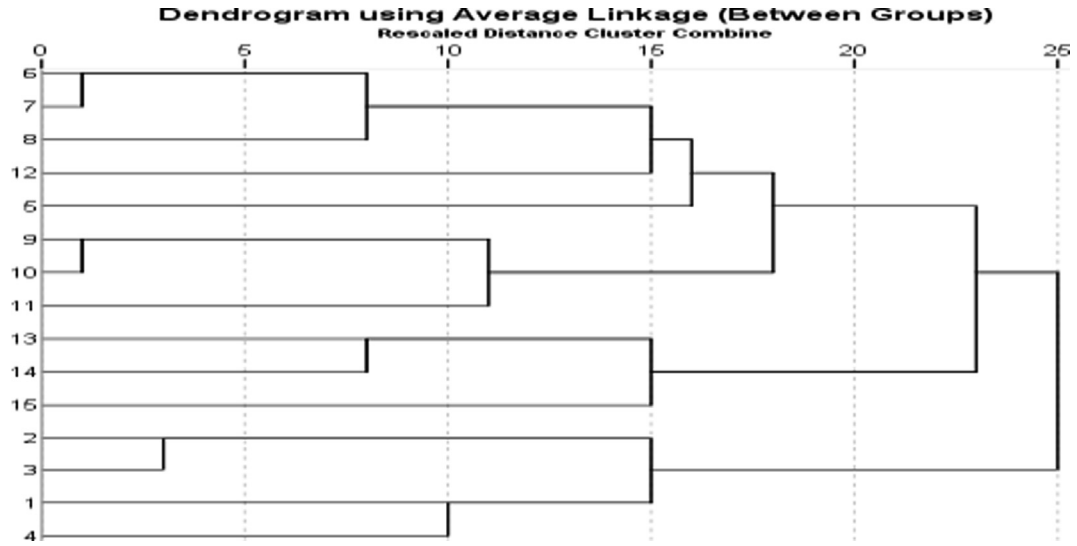

Fig. 5. Cluster tree illustrating the relationship among 15 alfalfa populations based on the analysis of 10 polymorphic SSR markers polymorphism, constructed utilizing the Euclidean similarity matrices computed as Dice coefficients and unweighted pair group method with arithmetic mean (UPGMA) algorithm in the SPSS software. Whereas, 1 = Wadi-1, 2 = Wadi-2, 3 = Wadi-3, 4 = Wadi-4, 5 = Wahat-1, 6 = Wahat-2, 7 = Wahat-3, 8 = Siwa-1, 9 = Siwa-2, 10 = Siwa-3, 11 = Promised pop., 12 = Nubaria, 13 = Rammah, 14 = Cuf-101 and 15 = Sea-reiver.

from cultivated populations to other ones, similar to Muller et al. (2001) and Falahati-Anbaran et al. (2007) endorsed previously. Our data depicted that SSR markers can be employed for population differentiation. The lowest coefficients value (0.42) of genetic uniformity was detected between Wahat-1 and Wadi-3, followed by (0.43) between Wahat-1 and Rammah. The highest coefficients value (0.92) of genetic similarity, on the other side, was detected between Wahat-3 and Wahat-2, followed by (0.90) between Siwa-2 and Siwa-3 (Table 8). A dendrogram was created relying on the assessed Dice coefficients by 98 polymorphic bands (Fig. 5). All the 15 alfalfa populations were clustered in two main clusters. The first one gathered all the Wadi's populations together, and the second one contained the remaining populations and was divided into two sub-clusters. The first grouped Rammah, Cuf-101, and Sea-river together. However, the other cluster was divided into two sub-sub-clusters (Fig. 5).

It, therefore, appears that SSR markers will be a potent molecular tactic for estimating genetic diversity and characterizing the germplasm in tetraploid alfalfa.

3.7.2. SCoT analysis

Seven SCoT primers were utilized to assess genetic divergence among 15 alfalfa populations. All the 7 SCoT primers were informative and discriminated among the alfalfa population (Table 9 and Fig. 6). One hundred and one bands were created from 7 SCoT primers, averaging 14.4 bands per primer, 91 bands of them were polymorphic. The number of bands primer^{-1} has fluctuated from 5 (SCoT-2) to 37 (SCoT-6).

For germplasm differentiation, two loci possessed positive, unique polymorphisms, which characterized the promising pop. from the other populations, which could be linked to the resistance of alfalfa damping-off disease. Whereas, 3, 3, 2, 2, 1, 1, and 1 loci had positive, unique polymorphisms that separated Wahat-1, Wadi-4, Wahat-3, Sea-river, Siwa-1, Cuf-101, and Rammah, respectively, from the remaining populations (Table 9).

While regarding Wadi-1 had one positive and one unique negative polymorphism that separated them from the remaining populations. SCot 2 primer was an uninformative primer. Although it was a polymorphic primer with a low polymorphic percentage, it

Table 9
The SCoT primers used and their amplification outcomes

Primer	Primer sequence (5′→3′)	Total No. of amplified bands (TNB)	No. of polymorphic bands (NPB)	Polymorphic bands (PPB, %)	Specific markers	
					Positive unique markers (PUM)	Negative unique markers (NUM)
SCoT1	5′-CAACAA**TG**GCTACCACCA-3′	19	19	100	–**1** at Wahat-3 at 88 bp –**1** at Siwa-1 at 322 bp	–
SCoT2	5′-CAACAA**TG**GCTACCACCC-3′	5	1	20	–	–
SCoT3	5′-CAACAA**TG**GCTACCACCG-3′	7	5	71.4	–	–**1** at Wadi1 at 303.4 bp
SCoT4	5′-CAACAA**TG**GCTACCACCT-3′	11	8	72.7	–**1** at Sea-reiver at 823.27 bp	–
SCoT5	5′-CAACAA**TG**GCTACCACGA-3′	14	13	92.9	– **1** at Wahat-1 at 1129.27 bp –**1** at Sea-reiver at 800 bp	
SCoT6	5′-CAACAA**TG**GCTACCACGC-3′	37	37	100	–**2** at Promised pop at 1230.88 and 286.57 bp –**1** at Wahat-3 at 167.56 –**1** at Wahat-1 at 112.85 bp	
SCoT7	5′-CAACAA**TG**GCTACCACGG-3′	27	27	100	–**1** at Wadi1 at 1500 bp –**3** at Wadi-4 at 1300, 1085.06 and 262.19 bp –**1**at Wahat-1 at 726.60 bp –**1** at Cuf-101 t 613.27 bp –**1** at Rammah at 404.59 bp	

couldn't distinguish between all used alfalfa populations (Table 9 and Fig. 6).

The Dice's similarity coefficients and UPGMA algorithm computed the 15 alfalfa populations based on the 7 SCoT markers. The coefficients of genetic similarity were ranged from 0.06 to 0.56. The lowest coefficients value (0.06) of genetic similarity was identified between Wahat-1 and Siwa-3 and between Wadi-4 and both of promising pop. and Nubaria. In contrast, the highest coefficient value (0.56) of genetic similarity was noticed between Wahat-2 and Wahat-3 (Table 10).

A dendrogram was created relying on the assessed Dice's coefficients by 91 polymorphic bands by unweighted pair group method of arithmetic averages (UPGMA), grouped 15 alfalfa populations in two main clusters, which branched more into subclusters (Fig. 7). The first main cluster gathered the Wadi-1, Siwa-3, Promising pop, and Nubaria. The second one contained the remaining populations and was divided into two subclusters, the first group Wadi-2, Wadi-3, and Wadi-4 together in a sub-sub cluster. In contrast, the other sub-sub cluster contained Wahat-2 and Siwa-2. At the same time, the other sub-cluster grouped the remaining alfalfa populations, as shown in Fig. 7.

4. Discussion

Results showed a positive relation between saponin concentrations and the reduction of mycelial growth of the tested fungi. *M. phaseolina* recorded a maximum reduction. The toxic influences of saponins are because of their capacity to form complexes with sterols of cell membranes, which leads to a loss of the integrity of these membranes (Bowyer et al., 1995).

The inhibitory effects of saponins have been reported. In this respect, Leath et al. (1972) reported a proven relationship between saponin and alfalfa pathogenic fungi growth reduction. Also, Omar et al. (1996) indicated that saponin significantly inhibited mycelia growth and reduced the sclerotial number of *Sclerotium cepivorum* (the causal organism of the white root of onion). Recently, Abd El-Rahman et al. (2018) found a significant reduction in mycelia growth and sclerotial formation of *Sclerotium rolfesii* isolates in response to saponin concentrations. Also, Omar (2019) showed antimicrobial properties of aerial parts of alfalfa saponin against some fungi and bacteria.

Our results showed considerable variation among alfalfa populations in their reaction against soil-borne fungi. Similarly, Anderson et al. (2013) and Abd El-Naby et al. (2014) indicated that cultivars and alfalfa populations differed in response to all fungi examined. The results showed higher performance of local tested populations than the other exotic ones overall studied traits *viz* plant height, number of tiller m^2, fresh and dry weight plant^{-1} and per m^2. Promised pop., Whahat −3, CuF-101, and Siwa-3 performed the highest fresh and dry yield (g) per plant and unit area of m^2. Selection for the high resistant plants over soil-borne fungi may be the best choice to improve alfalfa populations in the breeding program. These findings agree with those obtained by Abd El-Naby et al. (2014) and Anderson et al. (2013). Moutray (2000) reported that selection for grazing, frequent cutting elevated *Phoma* crown rot resistance, diminished blue alfalfa aphid resistance, and minimal alterations in the levels of resistance to pests and other diseases were occurred. Selection for fungal root rot resistance in individual plants per population was always predictive of a new elite blended population related to yield vigor.

Estimation of dry root samples of alfalfa tested populations revealed differences in saponin content. The resistant one recorded the maximum amount of saponin content /root dry mater. In previous studies (Fisher et al., 2018; Podolak et al., 2010), saponin is

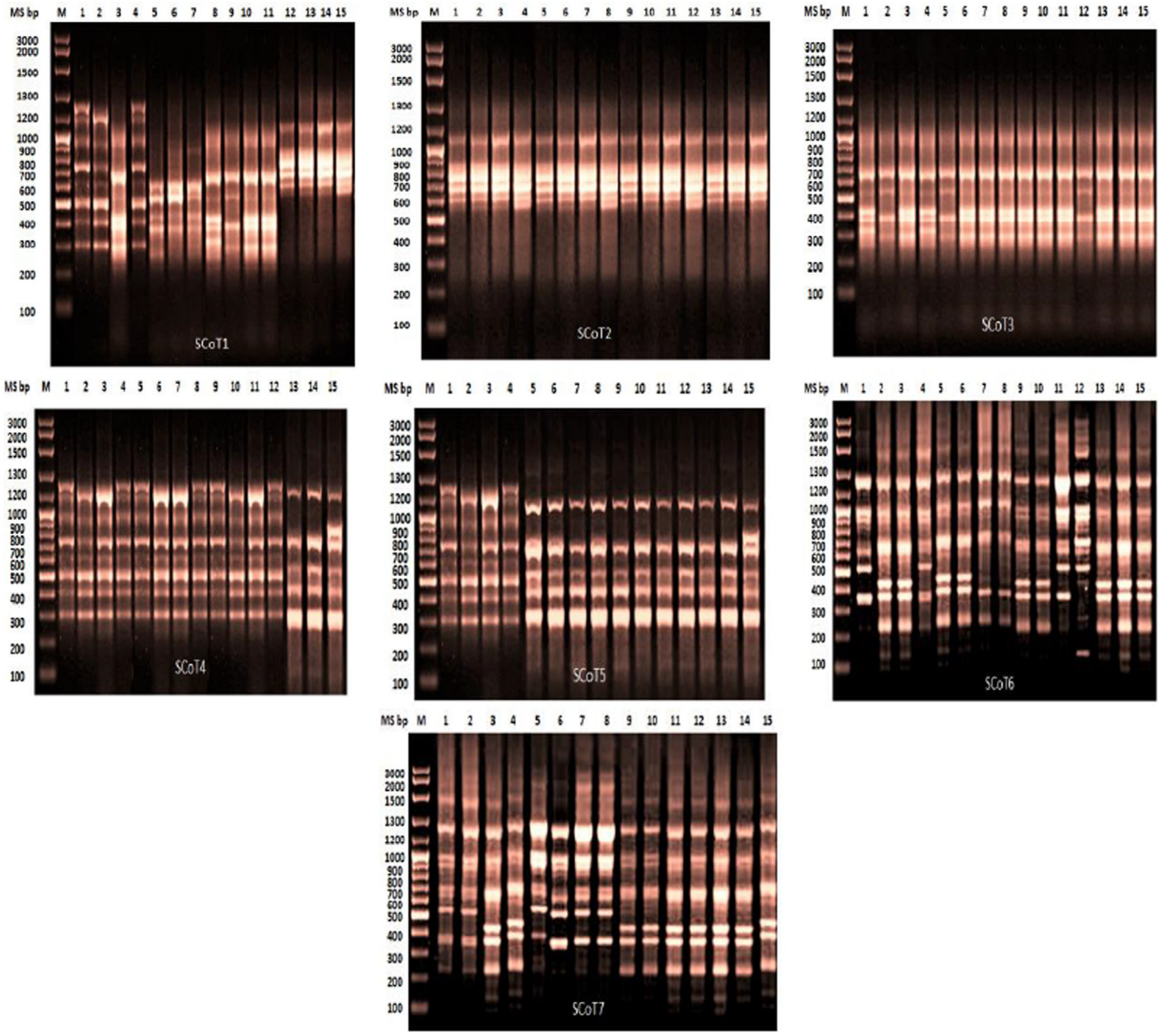

Fig. 6. DNA polymorphism of 15 alfalfa populations amplified using 7 SCoT primers. The letter 'M' denotes the molecular marker, Whereas, 1 = Wadi-1, 2 = Wadi-2, 3 = Wadi-3, 4 = Wadi-4, 5 = Wahat-1, 6 = Wahat-2, 7 = Wahat-3, 8 = Siwa-1, 9 = Siwa-2, 10 = Siwa-3, 11 = Promised pop., 12 = Nubaria, 13 = Rammah, 14 = Cuf-101 and 15 = Sea-reiver.

Table 10
Dice coefficient genetic similarity matrix among 15 alfalfa populations based on the 7 SCoT markers.

Alfalfa Pop.	1	2	3	4	5	6	7	8	9	10	11	12	13	14
2	0.34													
3	0.23	0.38												
4	0.11	0.40	0.49											
5	0.26	0.32	0.35	0.24										
6	0.17	0.11	0.22	0.20	0.24									
7	0.21	0.22	0.36	0.19	0.34	0.56								
8	0.20	0.20	0.43	0.17	0.16	0.29	0.38							
9	0.19	0.33	0.41	0.22	0.15	0.27	0.21	0.48						
10	0.17	0.17	0.22	0.13	0.06	0.20	0.25	0.17	0.22					
11	0.34	0.20	0.29	0.06	0.16	0.11	0.22	0.20	0.24	0.40				
12	0.20	0.20	0.29	0.06	0.11	0.11	0.11	0.25	0.29	0.34	0.40			
13	0.28	0.11	0.22	0.07	0.24	0.33	0.31	0.17	0.16	0.20	0.29	0.23		
14	0.30	0.21	0.29	0.18	0.32	0.24	0.28	0.26	0.29	0.12	0.36	0.31	0.29	
15	0.16	0.27	0.41	0.31	0.29	0.25	0.29	0.27	0.31	0.25	0.16	0.27	0.25	0.44

Whereas, 1 = Wadi-1, 2 = Wadi- 2, 3 = Wadi-3, 4 = Wadi-4, 5 = Wahat-1, 6 = Wahat-2, 7 = Wahat-3, 8 = Siwa-1, 9 = Siwa-2, 10 = Siwa-3, 11 = Promised pop., 12 = Nubaria, 13 = Rammah, 14 = Cuf-101 and 15 = Sea-reiver.

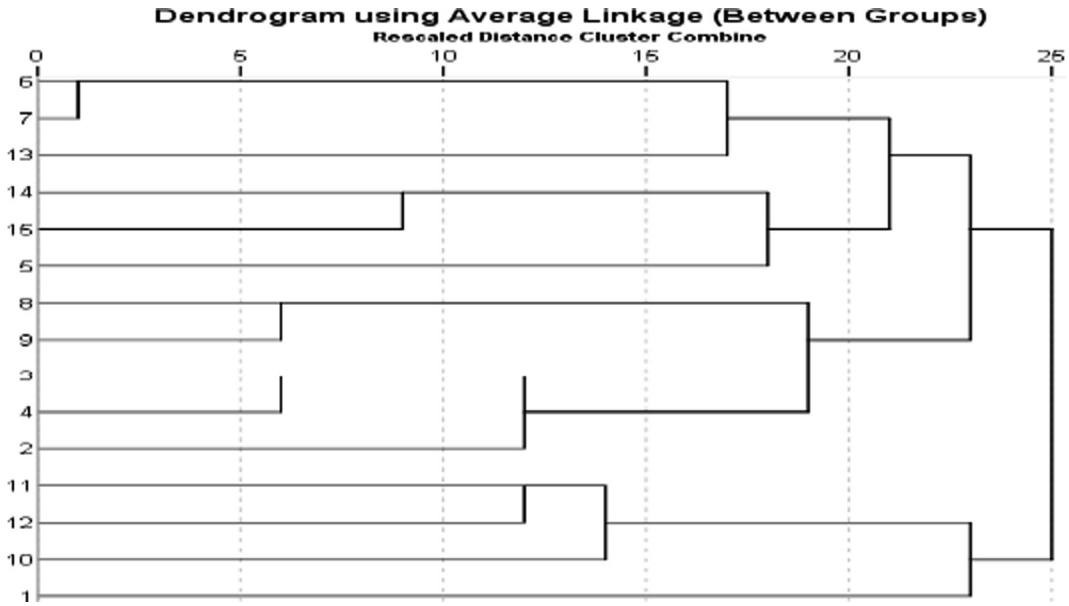

Fig. 7. Cluster tree illustrating the relationship among 15 alfalfa populations based on the analysis of 7 polymorphic SCoT markers polymorphism, constructed using the Euclidean similarity matrices computed as Dice coefficients and unweighted pair group method with arithmetic mean (UPGMA) algorithm in the SPSS software. Whereas, 1 = Wadi-1, 2 = Wadi- 2, 3 = Wadi-3, 4 = Wadi-4, 5 = Wahat-1, 6 = Wahat-2, 7 = Wahat-3, 8 = Siwa-1, 9 = Siwa-2, 10 = Siwa-3, 11 = Promised pop., 12 = Nubaria, 13 = Rammah, 14 = Cuf-101 and 15 = Sea-reiver.

classified as phytoalexin that causes disease resistance in plants against plant pathogens.

Also, lignin content in alfalfa tested populations markedly differed. Maximum content was more pronounced in the resistant ones. In this respect, Sticher et al. (1997) indicated that the lignin incorporation into the cell wall of a plant mechanically strengthens it, raising its resistance against degradation by enzymes of invading pathogens.

The crude protein content (%) is ordinarily utilized to measure the quality of the forage feed. Usually, the high quality of the forage feed is associated with the high protein content (McCoy and Walker, 1984; Van Saun, 2017). This study documented a converse relationship exists between yield and quality.

The genetic variation analysis of the breeding forage species *viz* alfalfa is a decisive step for identifying the genotype, analyzing the seed purity, and managing the germplasm. Improving forage quality using conventional and molecular tools is a recurrent breeding objective that should be prioritized.

This investigation also confirmed that SSR markers are valuable in estimating genetic relationships between alfalfa populations and identifying populations. It thus can be employed to identify duplicate accessions, manage conserved germplasm, assess seed purity in alfalfa populations, and protect the rights of plant breeders. Our findings are consistent with those in (Falahati-Anbaran et al., 2007).

In brief, 98 new genomic SSRs resulted from alfalfa, which has an outstanding advantage for evaluating polymorphic with possible use for studying genetic and phylogenetic mapping utilizing alfalfa, their benefit in characterization among the accessions to assess diversity will be strongly affected by the analyzed population nature.

SCoT marker is produced from the functional section of the genome, the genetic investigation utilizing this marker for crop development programs, *viz* genotype documentation, given the genetic diversity, creation of linkage maps, and QTL mapping (Collard and Mackill, 2009; Xiong et al., 2009; Hamidi et al., 2014; Abdein et al., 2018). SCoT markers are advantageous in genetic variation assessment due to their high reproducibility and the main perspective to reveal the polymorphism (Hamidi et al., 2014).

A major effect on crop improvement info was the dissemination and the degree of genetic variability and relationships among breeding materials. Based on our results, the SCoT marker, like the ISSR marker, was an actual method to evaluate the genetic variation. Furthermore, the tremendous polymorphic fragment percentage and number of polymorphic bands gained in our investigation specify the supremacy of SCoT marker in fingerprinting and diversity exploration. Likewise, our findings displayed a wide genetic variability among alfalfa genotypes that could be employed in alfalfa breeding programs. Thus, plant pathologists and breeders must focus on assembling more landrace populations, in addition to earning further genetic info for the improvement of new cultivars.

In short, breeding for saponin concentration in alfalfa plants may affect disease resistance to root rot and damping-off diseases. Such saponin concentration might improve plant growth, yield, and nutritional values.

5. Conclusions

The present study's finding showed a positive relation between saponin concentrations and the reduction of mycelial growth of the tested fungi. *M. phaseolina* recorded a maximum reduction. Our results showed considerable variation among alfalfa populations in their reaction against soil-borne fungi and significant variation among alfalfa populations in their response against soil-borne fungi. Estimation of dry root samples of alfalfa tested populations revealed differences in saponin content. The resistant one recorded the maximum amount of saponin content /root dry mater. Usually, the high quality of the forage feed is associated with the high protein content. A significant effect on crop improvement info was the dissemination and the degree of genetic variability and relationships among breeding materials. Based on our results, the SCoT marker, like the ISSR marker, was an actual method to evaluate the genetic variation. We could conclude that breeding saponin concentration in alfalfa plants may affect disease resistance to root rot and damping-off diseases. Such saponin concentration might improve plant growth, yield, and nutritional values.

6. Ethical disclosures

The authors announce that no experiments were performed on animals, and no data were collected from a patient in this research.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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