Assessment of the genetic variation in alfalfa genotypes using SRAP markers for breeding purposes

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ABSTRACT

The molecular diversity studies of alfalfa (*Medicago sativa* L.) germplasm could contribute to a more precise selection of parental populations in many breeding programs. Sequence-related amplified polymorphism (SRAP) markers were used to assess the genetic diversity of 110 individual plants from 13 selected alfalfa cultivars, landraces, and natural populations from Tunisia, Australia, Serbia, and Kazakhstan. Ten polymorphic SRAP primer combinations generated 137 alleles with 0.90 polymorphism information content. The percentage of polymorphic bands per genotype ranged from 57.66% to 70.07% with a mean of 64.29% and overall value of 100%. The genotype Sardi 10 had the highest value for the effective number of alleles; Nei's gene diversity and Shannon information index, exhibited the highest variability level (Ne = 1.453, He = 0.259, I = 0.381, respectively), whereas the genotype Nera exhibited the lowest variability level (Ne = 1.359, He = 0.211, I = 0.317, respectively). The AMOVA analysis showed that 68% of the variance was within the genotypes; this was in line with the coefficient of genetic differentiation (Gst = 0.370). The genetic relatedness of alfalfa individuals analyzed by the neighbor-joining dendrogram was consistent with the Bayesian model-based clustering approach. The exceptions were individuals from genotypes Slavija and Nera, which were grouped separately by STRUCTURE analyses. These results provide useful information for the management of alfalfa genetic resources and the rational use of local and foreign alfalfa populations in breeding programs focused on the development of new, high-yielding cultivars more adapted to drought conditions in North Africa.

Key words: Alfalfa genotypes, breeding, genetic relationship, *Medicago sativa*, SRAP markers.

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INTRODUCTION

The perennial, autotetraploid ($2n = 4x = 32$), allogamous, seed-propagated plant (Veronesi et al., 2010) alfalfa (*Medicago sativa* L.) is a member of the Fabales order, *Fabaceae* family, *Trifolieae* tribe, and the genus *Medicago*. Nowadays, alfalfa is considered as one of the most widely grown forage legumes in the world, and its important nutritive value makes it ideal for livestock and dairy production (Veronesi et al., 2010).

In Tunisia, alfalfa is the most important forage crop, grown on more than 12 410 ha, and approximately 75% of the oasis area reserved for alfalfa (9720 ha) is in southern Tunisia (Tlahig et al., 2017). Farmers mainly cultivate native germplasm (Benabderrahim et al., 2009) because they produce their own seeds, but also frequently exchange seeds among themselves (Julier et al., 2010). These local landraces, called 'Gabssia', are highly salt-tolerant and widely adapted to southern Tunisia farming regions (Loumerem et al., 2008; Benabderrahim et al., 2009). Landrace populations have been replaced by high-yielding introduced cultivars over time. Unfortunately, this massive and uncontrolled introduction of modern cultivars has increased the erosion of genetic diversity, a gradual loss of performance by traditional populations, as well as their slow extinction (Benabderrahim et al., 2015). In the context of not losing the performance of the local germplasm, the main goals in many crops were the evaluation of their genetic diversity followed by the selection and introduction into local breeding programs (Nass et al., 2012). In the last few decades, a diverse range of local germplasm has been collected in Tunisia (Loumerem et al., 2008); several projects for the development of new synthetic, high-yielding drought- and salt-tolerant alfalfa cultivars have been launched (Loumerem et al., 2008; Benabderrahim et al., 2015).

To estimate genetic diversity, molecular marker techniques with high polymorphism and independence of environmental factors can be used to detect variability between populations at the DNA level. The genetic diversity of alfalfa has been evaluated with various molecular marker systems, including amplified fragment length polymorphism (AFLP) (Keivani et al., 2010), random amplified polymorphic DNA (RAPD) (Nagl et al., 2011), simple sequence repeat (SSR) (Sakiroglu et al., 2010), and sequence-related amplified polymorphism (SRAP) (Al-Faifi et al., 2013). Compared with other DNA markers, SRAP has a range of advantages, including cost-effectiveness, relatively small amounts of required template genomic DNA, and clear, rarely overlapping high-intensity bands (Li and Quiros, 2001).

The molecular diversity studies of alfalfa germplasm could contribute to a more precise selection of parental populations required for crossing in alfalfa breeding procedures (Milic et al., 2013). The comparison of genetic diversity among native and foreign alfalfa genotypes, particularly in regions that import alfalfa seeds, such as Tunisia, has only been evaluated based on agro-morphological characters (Benabderrahim et al., 2015). Therefore, the objectives of the present study were to assess the genetic diversity of local Tunisian and geographically distant alfalfa genotypes and estimate their genetic relationship using SRAP markers.

MATERIALS AND METHODS

Plant material and DNA extraction

A total of 110 individual samples were collected from 13 alfalfa genotypes (Table 1), including cultivars, breeding populations, and landraces of different geographic origins: Tunisia (3), the United States (3), Serbia (5), Australia (1), and Kazakhstan (1).

Total genomic DNA was extracted from young leaves according to the cetyltrimethylammonium bromide (CTAB) method (Somma, 2004). The concentration and quality of DNA samples were detected with a ScanDrop Nano-Volume Spectrophotometer (Analytik Jena, Jena, Germany).

SRAP analysis

Ten SRAP primers (Li and Quiros, 2001) were tested for their ability to prime polymerase chain reaction (PCR) amplification of selected alfalfa genotypes. Ten primer combinations, which showed consistently reproducible polymorphism, were selected and used for further analysis (Table 2).

Amplification reactions were performed in 25 µL PCR mixture containing 10x taq DNA polymerase buffer, 200 µM each dNTP, 0.2 µM each primer, 50 mM $MgCl_2$, 2 U *Taq* DNA polymerase (Invitrogen, Paris, France), and 30 ng DNA. The DNA amplification was performed in a TPROFESSIONAL TRIO thermocycler (Biometra, Göttingen, Germany) according to the procedure described by Li and Quiros (2001). The PCR products were separated by electrophoresis in 3% agarose gel with 0.5 × TBE buffer (Tris-Boric acid EDTA, pH = 8) and stained with ethidium bromide. The gel image was captured using the Gel Doc. 2000 image analysis system (Bio-Rad, Hercules, California, USA).

Table 1. Names, type, origin, samples, and abbreviation of alfalfa genotypes.

Genotypes	Type	Origin	Samples	Abbreviation
Gabes-2012	Landrace	Gabes, Tunisia	9	G2012
Gabes-2355	Landrace	Gabes, Tunisia†	9	G2355
Gabes-2014	Landrace	Gabes, Tunisia†	9	G2014
T92	Cultivar	California, USA‡	9	T92
T93	Cultivar	California, USA‡	8	T93
T102	Cultivar	California, USA‡	8	T102
Banat VS	Cultivar	IFVCNS, Serbia	9	Banat
NS Jelena	Cultivar	IFVCNS, Serbia	5	Jelena
NS alfa	Cultivar	IFVCNS, Serbia	9	NS alfa
Nera	Cultivar	IFVCNS, Serbia	8	Nera
Slavija	Breeding population	IFVCNS, Serbia	9	Slavia
Sardi10	Cultivar	HS, Australia‡	9	Sardi 10
Kazakhstan	Natural population	Kazakhstan	9	Kaz

IFVCNS: Institute of Field and Vegetable Crops, Novi Sad; HS: Heritage Seeds.
†Seeds provided by the National Institute of Agronomic Research of Tunisia.
‡Seeds provided by the National Agronomic Institute of Tunisia.

Table 2. Primer sequence of sequence-related amplified polymorphism (SRAP) markers used in the study.

Forward primers (F)	Nucleotide sequences
F9	5'-GTA GCA CAA GCC GGA CC-3'
F13	5'-CGA ATC TTA GCC GGC AC-3'
Me4	5'-CGA ATC TTA GCC GGA AT-3'
F10	5'-GTA GCA CAA GCC GGA AG-3'
F11	5'-CGA ATC TTA GCC GGA TA-3'
Reverse primers (R)	
R9	5'-GAC TGC GTA CGA ATT TCA-3'
R7	5'-GAC TGC GTA CGA ATT GAG-3'
Em2	5'-GAC TGC GTA CGA ATT CGG-3'
R14	5'-CGC ACG TCC GTA ATT AAC-3'
R8	5'-GAC ACC GTA CGA ATT GAC-3'

Data analysis

Clear bands were scored as absence '0' or presence '1' of bands in binary matrices used for statistical analysis. The polymorphism information content (PIC) was calculated for each primer as the measure of marker discrimination power and informativeness (Botstein et al., 1980). The genetic diversity of alfalfa genotypes was studied with the POPGENE version 1.32 program (Yeh et al., 1999), which measured the following parameters: number (NPB) and percentage (PPB) of polymorphic bands, observed (Na) and effective number of alleles (Ne) per locus, Shannon information index (I), gene diversity index (He), coefficient of gene differentiation (Gst), and gene flow (Nm). The analysis of molecular variance (AMOVA) was performed with the GenAlEx 6.5 software (Peakall and Smouse, 2006).

A dendrogram was constructed by the neighbor-joining (NJ) method from the DARwin v.6.0.12 program (Perrier and Jacquemoud-Collet, 2006) based on Jaccard's coefficient. The Bayesian model-based clustering algorithms implemented in STRUCTURE v.2.3.4 (Pritchard et al., 2000) were applied to analyze the population structure of the alfalfa collection under study. The correlated allele frequencies under an admixture model were applied with a burn-in period of 10^5 followed by 10^6 MCMC (Monte Carlo Markov Chain) replicates. Ten independent runs for each predefined number of groups, ranging from 2 to 9, were performed to confirm consistency across the runs. Structure results were analyzed via the Structure Harvester Web server (Earl and von Holdt 2012) to determine the most likely number of groups, as suggested by Evanno et al. (2005).

RESULTS

SRAP markers

Ten SRAP primer combinations generated 137 well-amplified bands ranging in size from 150 to 4000 bp (Table 3). The ability of primer combinations to generate SRAP

Table 3. Polymorphism of sequence-related amplified polymorphism (SRAP) primer combinations selected in the study.

Primer combination	PIC	NPB	PPB	Band size
			%	bp
Me4-Em2	0.905	12	100	150-3500
F9-Em2	0.896	10	100	200-3500
F13-R7	0.817	6	100	400-2500
F9-R9	0.904	11	100	150-4000
F13-Em2	0.873	8	100	200-3000
F10-R14	0.936	23	100	300-3000
F11-R9	0.932	19	100	200-3500
F8-R9	0.928	15	100	200-4000
F10-R8	0.942	18	100	200-2000
F13-R9	0.881	15	100	300-2500
Mean	0.901	13.7	100	150-4000

PIC: polymorphism information content; NPB: number of polymorphic bands; PPB: percentage of polymorphic bands.

fragments varied significantly with values of 6 for *F13-R7* to 23 for *F10-R14* and a mean of 13.7 fragments per primer combination. All generated bands were polymorphic and showed a high range of variability in the tested alfalfa genotypes. The PIC values varied in a relatively narrow range from 0.81 (*F10-R8* primer combination) to 0.94 (*F13-R7* primer combination) with a mean of 0.90 (Table 3).

Genetic diversity analyses

The assessment values of genetic variation between and within alfalfa genotypes based on SRAP markers are shown in Table 4. Although none of the fragments were monomorphic in all the tested genotypes, they were uniform in the same genotypes, while the level of polymorphism varied in others. The number and percentage of polymorphic bands, as well as observed number of alleles, were the highest in genotypes Banat and NS Alfa (NPB = 96; PPB = 70.07%, and Na = 1.701, respectively), while these parameters were the lowest (NPB = 79; PPB = 57.66%, and Na = 1.577, respectively) in genotype NS Jelena. The value of the effective number of alleles in tested genotypes ranged from 1.361 to 1.453 with a mean of 1.409 and an overall value of 1.662 among genotypes. The genetic diversity of the populations was measured by Nei's genetic diversity index and it ranged from 0.212 to 0.259 with a mean of 0.237 within genotypes and an overall value of 0.374 among genotypes. The Shannon information index ranged from 0.318 to 0.381 with a mean of 0.351 within genotypes and 0.548 among genotypes. Among the 13 studied genotypes, genotype Sardi 10 exhibited the highest level of variability (Ne = 1.453, He = 0.259, I = 0.381), whereas genotype Nera exhibited the lowest level of variability (Ne = 1.359, He = 0.211, I = 0.317).

The AMOVA results (data not shown) revealed that 68% of the molecular variation in tested alfalfa genotypes existed within populations and with 32% among populations. All the molecular variance components were highly significant ($P < 0.001$). Values for the coefficient of genetic differentiation (Gst = 0.370) showed a similar allocation of genetic diversity and a relatively small proportion of genetic diversity (37%) among genotypes as compared to 63% of genetic diversity within them. The gene flow (Nm) among genotypes was estimated to be 0.851 individuals per generation, indicating a relatively low gene flow among them.

Cluster analyses

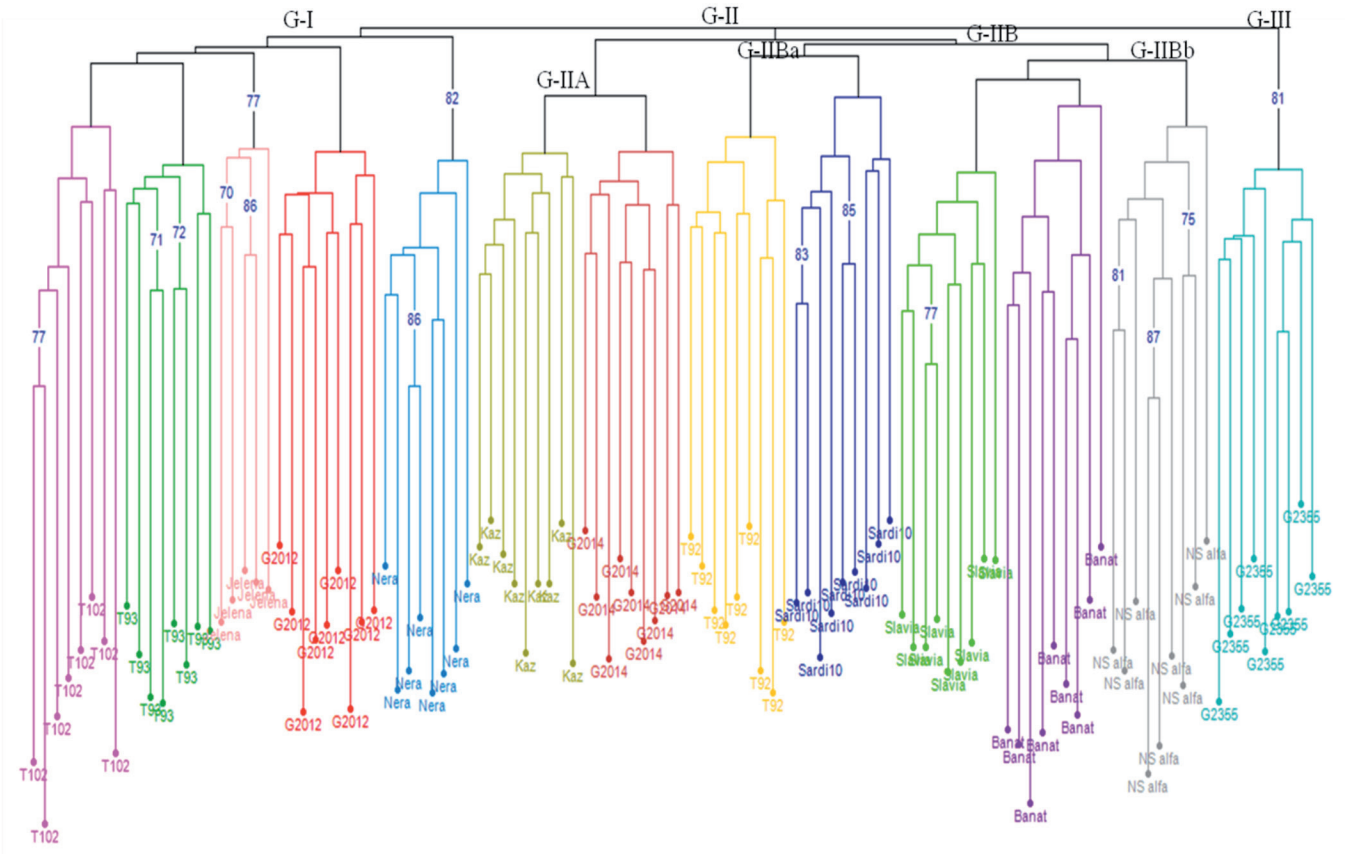
The neighbor-joining dendrogram, which was constructed based on the genetic distance matrix, showed that alfalfa genotypes were grouped into three major clusters (Figure 1). The first cluster (G-I) consisted of five genotypes (T102, T93, NS Jelena, G2012, Nera) of which genotypes T102 and T93 were grouped into a separate sub-cluster and then the rest joined them one by one. The last to join this cluster was genotype Nera with a high bootstrap value (86%). The second cluster (G-II) consisted of two sub-clusters. The first sub-cluster (G-IIA) consisted of genotypes Kaz and G2014. In the second sub-cluster (G-IIB), genotype T92 joined genotype Sardi 10 (G-IIBa) and then this group joined the (G-IIBb) group that clustered genotypes Slavia, Banat VS, and NS Alfa. The individuals from genotype G2355 were clearly (80% bootstraps) separated in a third cluster (G-III).

Table 4. Genetic diversity parameters of alfalfa genotypes revealed by sequence-related amplified polymorphism (SRAP) markers.

Genotypes	NPB	PPB	Na	Ne	He	I
T92	93	67.88	1.679 ± 0.467	1.410 ± 0.377	0.239 ± 0.198	0.357 ± 0.280
T93	87	63.50	1.635 ± 0.483	1.406 ± 0.374	0.236 ± 0.201	0.351 ± 0.286
T102	94	68.61	1.686 ± 0.466	1.445 ± 0.396	0.257 ± 0.199	0.381 ± 0.281
G2014	87	63.50	1.635 ± 0.483	1.424 ± 0.389	0.242 ± 0.206	0.357 ± 0.292
G2012	89	64.96	1.650 ± 0.479	1.391 ± 0.370	0.230 ± 0.196	0.345 ± 0.279
G2355	80	58.39	1.584 ± 0.495	1.370 ± 0.375	0.215 ± 0.204	0.320 ± 0.291
Sardi 10	91	66.42	1.664 ± 0.474	1.453 ± 0.386	0.259 ± 0.203	0.381 ± 0.288
Kaz	86	62.77	1.628 ± 0.485	1.423 ± 0.392	0.241 ± 0.208	0.355 ± 0.294
Slavija	83	60.58	1.606 ± 0.491	1.406 ± 0.390	0.232 ± 0.208	0.342 ± 0.296
NS Jelena	79	57.66	1.577 ± 0.496	1.390 ± 0.393	0.223 ± 0.208	0.328 ± 0.297
Banat VS	96	70.07	1.701 ± 0.460	1.409 ± 0.371	0.241 ± 0.193	0.362 ± 0.271
NS alfa	96	70.07	1.701 ± 0.460	1.428 ± 0.376	0.249 ± 0.197	0.371 ± 0.277
Nera	84	61.31	1.613 ± 0.489	1.361 ± 0.373	0.212 ± 0.199	0.318 ± 0.283
Average	88.08	64.29	1.643 ± 0.479	1.409 ± 0.382	0.237 ± 0.201	0.351 ± 0.286
Overall	137	100	2.000 ± 0.000	1.662 ± 0.303	0.374 ± 0.138	0.548 ± 0.174

NPB: number of polymorphic bands; PPB: percentage of polymorphic bands; Na: observed number of alleles; Ne: effective number of alleles; He: Nei's genetic diversity; I: Shannon information index.

Figure 1. Neighbor-joining dendrogram showing the genetic relationships between tested alfalfa genotypes based on sequence-related amplified polymorphism (SRAP) data.



Individuals were colored on the basis of genotype affiliation. The number at each node represents the bootstrap value (> 70%) based on 1000 bootstrap replicates. Genotype abbreviations: Gabes-2012: G2012; Gabes-2355: G2355; Gabes-2014: G2014; T92: T92; T93: T93; T102: T102; Banat VS: Banat; NS Jelena: Jelena; NS alfa: NS alfa; Nera: Nera; Slavija: Slavia; Sardi10: Sardi 10; Kazakhstan: Kaz.

The alfalfa data was further analyzed by a model-based clustering approach. The results showed that the maximum delta K was detected at K = 7 and the second delta K peak was detected at K = 2 (Figure 2). Given that the delta K peak at K = 2 may be an artifact (Vigouroux et al., 2008; Wang et al., 2014), the alfalfa data set was further divided into seven groups (Figure 3). Individuals with a membership coefficient less than 0.8 were considered as mixed; 105 individuals (95.45%) were assigned to one of the model's defined groups. The largest group (A) consisted of individuals from genotypes T102, T93, NS Jelena, and G2012 with a membership coefficient between 0.801 and 0.987. All the individuals from genotypes Kaz and G2014 were placed in group C with a membership coefficient greater than 0.85, except individual 9 from genotype G2014 that could be considered as admixed (coefficient 0.72). Group D included individuals from genotypes T92 and Sardi 10. Although individual 1 from genotype Sardi 10 could be considered as admixed, the highest proportion of the variation is attributed to this group (coefficient 0.70). With the exception of individual 3, all the samples from genotypes Banat and NS alfa were classified in group F with a membership coefficient greater than 0.85. Individuals from genotypes Nera and Slavia were assigned to separate groups (B and E, respectively), except individual 2 from both genotypes, which could be considered as admixed (membership coefficient 0.57 and 0.79, respectively). All individuals from genotype G2355 were grouped into one single group (with membership coefficient greater than 0.9), distinguishing them from individuals from all the other genotypes.

Figure 2. The most likely number of groups (K) based on delta K assumed in the STRUCTURE analyses.

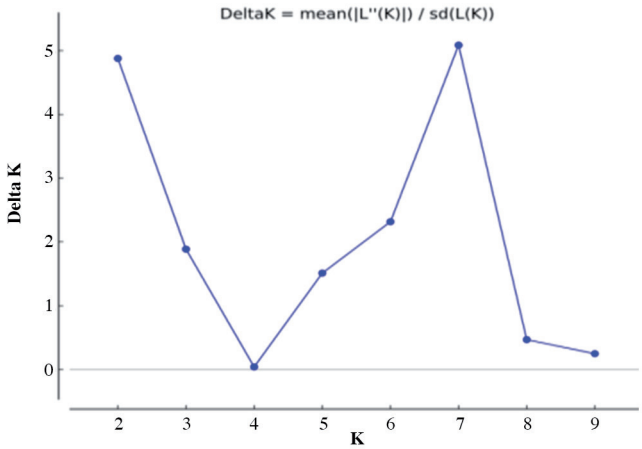
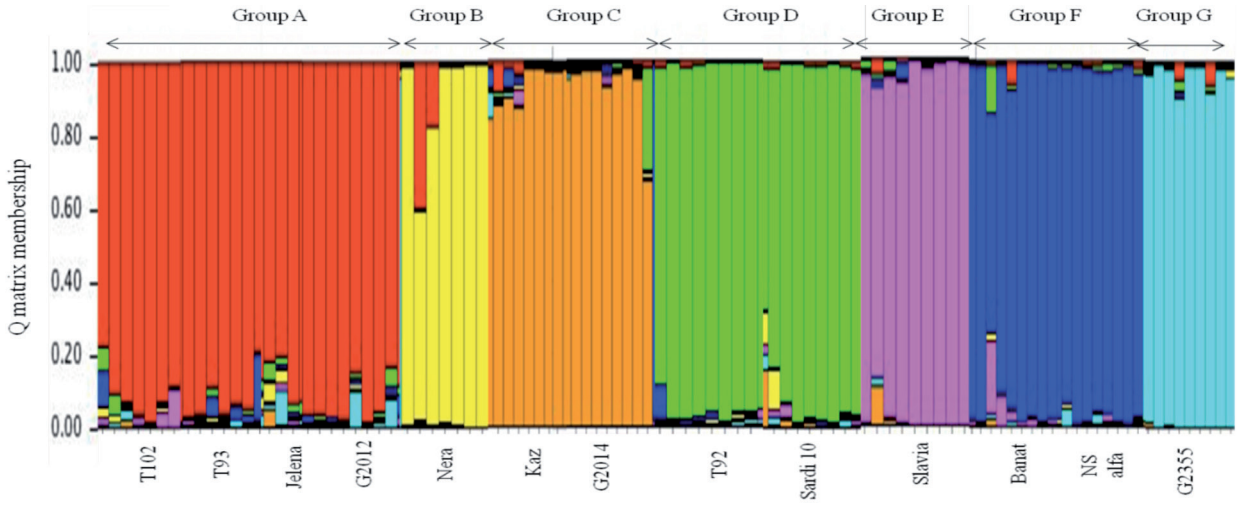


Figure 3. Bayesian structure analysis of 110 individuals from the 13 studied alfalfa genotypes based on sequence-related amplified polymorphism (SRAP) molecular markers.



Each individual is represented by a vertical line, which is colored according to the assigned groups at estimated K = 7. Genotype abbreviations: Gabes-2012: G2012; Gabes-2355: G2355; Gabes-2014: G2014; T92: T92; T93: T93; T102: T102; Banat VS: Banat; NS Jelena: Jelena; NS alfa: NS alfa; Nera: Nera; Slavija: Slavia; Sardi10: Sardi 10; Kazakhstan: Kaz.

DISCUSSION

The genetic erosion of alfalfa landraces in Tunisia emphasizes the need for their rapid assessment due to preservation and use in breeding. The genetic diversity level and patterns of population structure of *M. sativa* are required to understand the management of genetic resources in this species as well as for further progress in breeding programs. The breeding strategy is based on the partial exploitation of heterosis in alfalfa proposed for the development of semi-hybrids by crossing genetically divergent germplasm and identifying heterotic groups (Brummer, 1999; Milic et al., 2013; Annicchiarico et al., 2017). One potential source of a heterotic combination within an alfalfa combination would be crossing between genetically distant populations (Li and Brummer, 2012). Therefore, we assumed that local Tunisian germplasm, which has a long history of growing in the relatively isolated environment of the oasis, can be valuable heterotic populations in semi-hybrids with selected, geographically distant populations.

The percentage of polymorphic bands obtained in our study was 100%, suggesting very high genetic diversity in tested alfalfa genotypes. Previous genetic diversity studies using SRAP markers also showed a high polymorphism percentage in alfalfa, including 98.3% in the study of Saudi alfalfa landraces (Al-Faifi et al., 2013) and 90% in the study of 15 alfalfa populations (Vandemark et al., 2006). Moreover, the percentage of polymorphic bands was the highest value as compared to results obtained by Talebi et al. (2011) using 14 SRAP markers in Iranian alfalfa cultivars (PPB = 49%) and Yuan et al. (2011) using 34 SRAP markers in 26 Chinese alfalfa cultivars (PPB = 40.93%). This finding supports the idea that the number of amplified fragments depends on the genotypes used as well as primer combination (Tucak et al., 2008).

All SRAP markers exhibited high PIC values ranging from 0.81 to 0.94 with a mean of 0.90. Observed PIC values were within a relatively narrow range, indicating a uniform distribution of SRAP polymorphism across collected genotypes, which is a preferred characteristic for their use in genetic diversity analyses (Al-Faifi et al., 2013). High polymorphism of the selected SRAP markers offers a unique opportunity for studying the genetic variation and relationship of alfalfa germplasm. In general, genetic diversity parameters had high values, showing that variability of the tested population was high. A high diversity level in alfalfa was also reported using different marker systems (Taski-Ajdukovic et al., 2014). Such variability may improve the adaptation of alfalfa genotypes to a wide range of environments (Nagl et al., 2011).

The AMOVA showed a higher distribution of genetic variation within genotypes (68%) than among them (32%). This result is in accordance with previous alfalfa studies (Nagl et al., 2011; Talebi et al., 2011). This was expected because alfalfa is characterized by its allogamy, autotetraploidy, and strict outcrossing, which promotes the maintenance of large within-population diversity and in this way hinders effective genetic variability among populations (Taski-Ajdukovic et al., 2014). Some authors have suggested that intra-population variability is mainly affected by evolutionary factors, such as the breeding system, gene flow, seed dispersal, as well as natural selection and ecological and geographical factors (Julier et al., 2010). In this context and as a consequence of high heterozygosity and heterogeneity, alfalfa cultivars are composed of thousands of plants of different genotypes (Vandemark et al., 2006).

The genetic relatedness of alfalfa individuals analyzed by the neighbor-joining dendrogram was consistent with the Bayesian model-based clustering approach with two

exceptions: a) genotype Nera was the last to join sub-cluster I (86% bootstraps), so its separation by another type of analysis could be expected, and on the contrary b) genotype Slavia was sub-clustered with small bootstrap values (19% bootstraps), and it is therefore not surprising that it is detached from this sub-cluster by STRUCTURE analyses. Both analyses clearly distinguished the landrace G2355 from all other tested germplasm (80% bootstraps and membership coefficient greater than 0.9). Offspring of that genotype could be superior to the other parental combinations in semi-hybrid breeding. The potential of heterosis in such semi-hybrids could be high because genotype G2355 was defined as highly adapted to oasis conditions (Khelifi et al., 2008). This genotype has a high forage yield potential, fast autumn re-growth, less sensitivity to parasites, and high leaf somatic resistance values under drought stress (Khelifi et al., 2008; Bouizgaren et al., 2010). Another landrace from Tunisia, G2014, and a genotype from Kazakhstan grouped in separate sub-clusters indicate that they are genetically distant and can also be a potential source for semi-hybrids. Genotypes Banat and NS Alfa and the breeding population Slavia from the Institute of Field and Vegetable Crops (IFVCNS, Novi Sad, Serbia) breeding program were grouped. This was expected because genotype Banat was selected from local Pannonian populations while NS Alfa and Slavia have a genetic background that is mostly from West European alfalfa populations (Katic et al., 2008). Not including other genotypes (Jelena and Nera) in the group may be due to specific population traits and pedigree. Genotype Jelena originates from crosses between Greek and Serbian populations; along with Greek and Serbian origins, genotype Nera also has a genetic background from central European and Iranian alfalfa populations (Katic et al., 2008), suggesting its detachment by STRUCTURE analyses. Genotypes Nera and NS Jelena belong to dormancy group 6; they currently represent most non-dormant alfalfa cultivars from the IFVCNS breeding program, are drought-tolerant, and also intended for growth in Mediterranean regions. Their grouping in the same cluster with local G2012 and introduced genotypes (T92 and T93) suggested that they can adapt to regions such as North Africa; they will be tested under field conditions in Tunisia to be included in breeding programs. Both American and Australian cultivars were introduced in Tunisia; these cultivars surpassed the productivity of the local Gabes landrace and were the object of multiplication programs to provide more seeds for alternative use by regional farmers (Hayek et al., 2008). The massive and uncontrolled introduction of commercial varieties is a serious threat to the genetic diversity of traditional populations (Benabderrahim et al., 2015). Clustering Gabes landrace with introduced genotypes suggested that there was a gene flow between them or that they adapt well in this type of environment and thus have similar characteristics, such as high drought and salt tolerance levels, and belong to close dormancy classes (moderately dormant or non-dormant populations). Massive intercrossing of the local Tunisian genotypes collected from different Tunisian oasis environments with some introduced cultivars has been carried out (Benabderrahim et al., 2015). A new breeding program has been established aimed at developing salt- and drought-tolerant cultivars. Introduced germplasm (foreign cultivars in Tunisian arid regions), including the Australian variety Sardi10, were used as parental populations in this project (Loumerem et al., 2008).

CONCLUSIONS

Results confirmed that sequence-related amplified polymorphism (SRAP) markers can be effectively used to estimate genetic diversity among tested alfalfa genotypes. This study provides valuable data for the identification and rational use of local and foreign alfalfa populations. It can significantly contribute to current breeding programs focusing on the development of new, high-yielding cultivars that are more adapted to the drought conditions in North Africa. The local landrace G2355 was genetically the most distant, so that semi-hybrids formed by hybridizing them could be superior to other parental combinations. The potential of high heterosis in these semi-hybrids may be accomplished given the high adaptation of this genotype to oasis conditions. The most homogeneous genetic material among the examined alfalfa genotypes (Nera, NS Jelena) will be used for further analyses to check agronomic potential in terms of yield, perenniality, and persistence in the target regions.

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