

## ASSESSMENT OF THE GENETIC DIVERSITY BETWEEN TWO VARIETIES OF WONDERBOOM (*FICUS SALICIFOLIA* VAHL.) BY USING SIMPLE SEQUENCE REPEAT (SSR) MARKERS

AMEDJKOUH, H.<sup>1,2\*</sup> – BOUGUEDOURA, N.<sup>1</sup>

<sup>1</sup>Research Laboratory of Arid Zones, Faculty of Biological Sciences, University of Sciences and Technology Houari Boumediene (USTHB), PO Box 113, El Alia 16111, Bab-Ezzouar, Algiers, Algeria

<sup>2</sup>Department of Biology of Populations and Organisms, Faculty of Sciences of Nature and Life, University of Blida I, PO Box 270, Soumaa Road, Blida, Algeria

\*Corresponding author

e-mail: hafida.lrza@gmail.com; phone: +213-771-029-173

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**Abstract.** This study aims to determine the genetic diversity of *Ficus salicifolia* varieties collected from (Tamanrasset) Southern Algeria. Currently, little is known about the genetic variability between two varieties “Teloukat” and “Eucalyptoïdes”. Thirty-nine fig trees were examined by using six simple sequences repeat (SSR) markers. In total 64 alleles were observed in the samples of two varieties. The number of alleles per locus ranged from seven to eighteen, with an average of 10.66 alleles per locus. The mean values of heterozygosities observed and expected were 0.61 and 0.74 respectively. The positive Fis (Wright inbreeding coefficient) value obtained with (MFC2, MFC8, LMFC26, LMFC30 and FSYCO1) loci indicated deficiency of heterozygote. Considering the PIC (polymorphic information content) results, 5 markers (MFC2, MFC8, LMFC26, LMFC30 and FSYCO1) were classified as highly informative (PIC > 0.5). The phylogenetic diagram performed using the Unweighted Pair Grouping Method with Arithmetic average (UPGMA) showed significant intraspecific polymorphism between the two varieties which was confirmed by Factorial Correspondence Analysis (FCA).

**Keywords:** *Ficus salicifolia*, Tamanrasset, SSR markers, heterozygote, UPGMA, intraspecific polymorphism

### Introduction

*Ficus* (figs) is a genus which includes economically important fruit species (*Ficus carica*), present worldwide, with 750 species in total from *Ficus* genus occurring throughout tropical and subtropical regions, almost of which are evergreen trees, shrubs or lianas producing the latex and enclosed inflorescences (Berg, 1989).

In Southern Algeria (Tamanrasset), the *Ficus* genus is commonly represented by *Ficus salicifolia* Vahl., an endemic species of central Sahara (Ozenda, 1977), which is highly fragmented and rarely exceeds 30 individuals making them locally endangered. It develops intermediate growth forms between shrubs and tree, with small (10 to 20 mm diameter) ripe dark red figs which are edible for humans and animals, two varieties are cultivated “variety Teloukat” and “variety Eucalyptoïdes” (Battandier and Trabut, 1912). Both grow at high altitudes (1400 to 2700 m) along Rockies flaws ravines under arid conditions (temperature from 16 to 30 °C, rainfall less than 50 mm per year).

Several homonyms and synonyms of these trees are used by aboriginals, which make their characterization very difficult, because there is not yet real recognition or information on their ecological or genetic divergence (Sahki and Sahki, 2004). Currently, little is known about genetic diversity of *Ficus salicifolia* varieties except

morphological characters description which are still unreliable for the unambiguously identification of two varieties (Teloukat and Eucalyptoïdes). In addition, variation in phenotypic characters does not necessarily reflect the genetic diversity of the population (Bagavathiannan et al., 2010). Further, most morphological traits are influenced by environmental factors (Teoman et al., 2017). In this investigation, we refer to groups of trees in natural habitats to assess their genetic diversity.

Since, objectivity is essential for the identification; morphological traits can be unreliable for genetic plant identification and characterization (Ercisli et al., 2008). To preserve and improve existing plant genetic resources under harsh conditions, it will be helpful to evaluate genetic diversity and molecular markers permits a more rapid and reliable approach, so, they offer numerous advantages over conventional alternatives based on morphological traits; these markers are stable and detectable in all plant tissues, regardless of environmental conditions (Leal et al., 2010; Do Val et al., 2013).

Previous studies have showed that DNA marker technologies provided an effective tool for the conservation of fig genetic resources, such as random amplified polymeric DNA (RAPD) (Dalkilic et al., 2011; Baziar et al., 2018), restriction fragment length polymorphism (RFLP) (Khadari et al., 2005), amplified fragment length polymorphism (AFLP) (Baraket et al., 2011) and single sequence repeat (SSR) (Ferrara et al., 2016; Fu et al., 2017). To study the genetic diversity, the SSR markers were used regarding their highly polymorphic, co-dominant and abundance in the genome (Fu et al., 2017; Hladnik et al., 2018). Microsatellite markers have been widely used for the analysis of genetic diversity and they play an essential role in all aspects of plant selection; as: apple (*Malus domestica*) (Pikunova et al., 2018); apricot (*Prunus armeniaca*) (Köse et al., 2017); peach (*Prunus persica*) (Licea-Moreno et al., 2019); olive (*Olea europaea ssp europaea*) (Ben Mohamed et al., 2017) and (*Olea europaea ssp laperrinei*) (Baali-Cherif and Besnard, 2005). SSR markers are also widely used for the evaluation of the diversity studies and characterization of Fig species trees (Khadari et al., 2004; Essid et al., 2015; Boudchicha et al., 2018).

The apparent ambiguity in the taxonomic classification of two varieties not distinct morphologically (Teloukat and Eucalyptoïdes), directed us to the use of molecular markers; therefore, the aim of this present study is to determine the genetic diversity within individuals of *Ficus salicifolia* enabling to establish the relationship between the two varieties. On the other hand, to define a conservation strategy, the genetic diversity of this species has to be investigated.

## Materials and methods

### *Plant material*

Leaves samples of thirty-nine individuals of *Ficus salicifolia* (Fig. 1) of both varieties: (Eucalyptoïdes and Telloukat) were collected randomly from a natural habitat of six zones (Table 1; Appendix 1; Fig. 2) located in Ahaggar (Tamanrasset: 22°57'42" north, 5°11'51" east) in the central Sahara of Southern Algeria.

### *Molecular markers*

#### *DNA extraction*

Total genomic DNA was extracted as described by Khadari et al. (2004) using 200 mg of leaves dried in silica gel, according to the DNeasy Plant Mini Kit (QIAGEN,

Courtaboeuf, France) optimized by adding 1% polyvinylpyrrolidone (PVP 40.000) to the buffer AP1.

DNA was quantified using spectrometric method (Sambrook et al., 1989): the absorbance was recorded at 260 nm wavelength (Spectrophotometer UV mini-1240, Shimadzu Japan). The relative purity of extracted DNA was estimated after electrophoresis with 0.7% agarose gel stained by 0.2 µg.l<sup>-1</sup> Ethidium Bromide and immersed in 90 mM Tris-Borate, pH 8 and 10 mM Ethyldiamine Tetra-acetic Acid (EDTA), the DNA samples were visualized under ultraviolet light.

### SSR markers

The 6 SSR markers were selected due to their polymorphism level, the high quality of amplification reproducibility and transferability, since five SSR markers were developed for a common fig (*Ficus carica* L): LMFC26, LMFC30 (Giraldo et al., 2005), MFC2 (Khadari et al., 2001) and MFC8, MFC12 (Achtak et al., 2009), and a marker FSYCO1 was developed for *Ficus sycomorus* (Ahmed et al., 2007). The details of each marker are shown in Table 2.

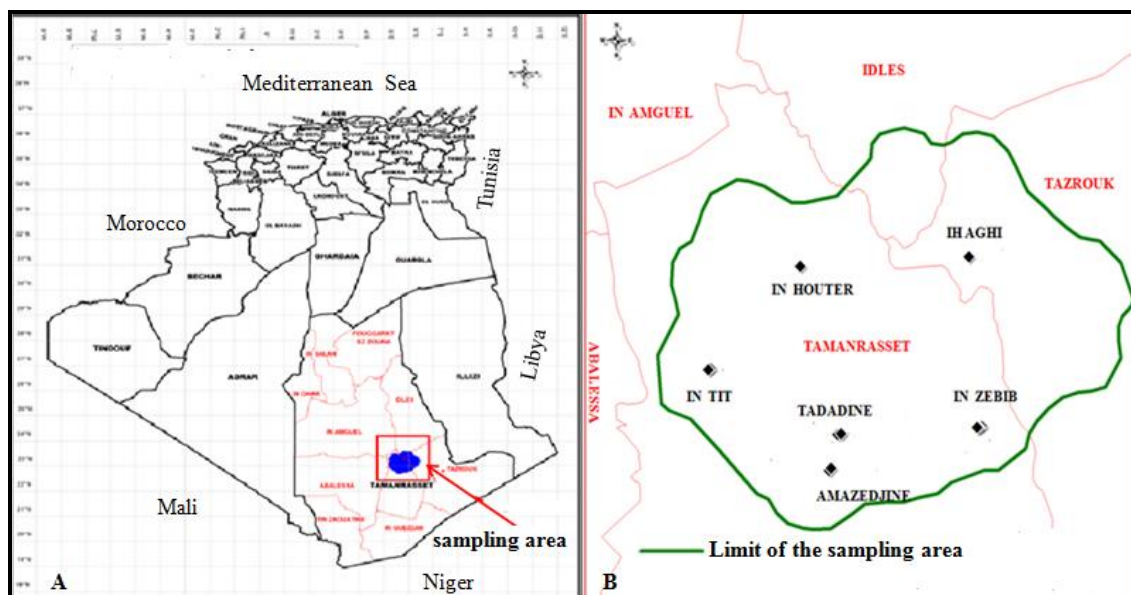


**Figure 1.** Morphology of *Ficus salicifolia* in arid zones (Tamanrasset). A: External morphology of the tree. B: morphological appearance of some vegetative (leaves, shoots) and reproductive (fruits) parts

**Table 1.** List of individual fig trees with GPS data

| Local name of collection zones | Individuals number | Code of trees  | Altitude (m)      | Latitude/longitude  |
|--------------------------------|--------------------|--|-------------------|---------------------|
| TIT                            | 10                 | from Fs01 to Fs10<br>Variety Teloukat                              | From 1081 to 1088 | 22°57'46"/05°09'76" |
| IH-AGHI                        | 05                 | from Fs11 to Fs15<br>Variety Teloukat                              | From 1093 to 1095 | 23°04'10"/05°12'85" |
| IN-HOUTER                      | 01                 | Fs16<br>Variety Eucalyptoides                                      | 1455              | 23°04'73"/05°22'85" |
| TADADINE                       | 03                 | from Fs17 to Fs19<br>Variety Eucalyptoides                         | From 1315 to 1350 | 22°49'42"/05°57'33" |
| IN-ZEBIB                       | 18                 | from Fs20 to Fs37<br>Variety Teloukat and<br>Variety Eucalyptoides | From 1425 to 1459 | 22°48'36"/05°37'49" |
| AMEZEDJINE                     | 02                 | from Fs38 to Fs39<br>Variety Teloukat                              | From 1162 to 1165 | 22°36'96"/05°24'46" |

Fs, *Ficus salicifolia*



**Figure 2.** Map showing location of sampling areas, in Tamanrasset (southern Algeria). A: Algeria within north Africa on a globe (www.Alamy.com). B: Stations of collection

**Table 2.** Fig SSR primer sequences used

| Microsatellite primers | Repeat motif   | Primer sequences 5' to 3' (F) (Forward) | Primer sequences 5' to 3' (R) (Reverse) | Tm (°C) |
|------------------------|--|---|---|---------|
| MFC2                   | (AC) <sub>18</sub> (AT) <sub>7</sub>                     | GCTCCGATGCTGCTCTTA                      | TCGGAGACTTTTGTTC AAT                    | 55      |
| FSYCO1                 | (A) <sub>16</sub> (GAAA) <sub>8</sub>                    | CAAATGAAAAACACAAATTTGCCAA               | TGCAAGTACTAATTCCTCTGCCGTG               | 55      |
| LMFC26                 | (TC) <sub>11</sub>                                       | ATGTTATAGTTGAGTGAGGATAA                 | AAATAGTGGATCTTGCATGT                    | 55      |
| MFC8                   | (CA) <sub>9</sub> TA(CA) <sub>14</sub> (TA) <sub>6</sub> | GTGGCGTCGTCTCTAATAAT                    | TATTCTATGCTGTCTTATGTCA                  | 50      |
| MFC12                  | /  | TATCACGGCGGTCTAACTCTGC                  | CTCCTCATCCCCCTCCCAACT                   | 50      |
| LMFC30                 | (CT) <sub>14</sub>                                       | TTGTCCGTTTCTTATACAAT                    | TCTTTTAGGCAGATGTTAG                     | 55      |

### PCR amplification and genotyping procedure

Amplification was carried out using the PCR conditions of fig studies (Khadari et al., 2004; Achtaq et al., 2009) by conducting polymerase chain reaction (PCR) amplification in a total volume of 20 µL containing PCR buffer [10 mM Tris-HCl (pH 8.3), 50 mM KCl, 0.1% Triton X-100 (Perkin Elmer, Milan, Italy) and 0.02% gelatin], 20 ng of fig genomic DNA, 2 mM MgCl<sub>2</sub>, 0.2 mM of each dNTP, 1 to 4 pmol for dye-labeled primers [fluorescent phosphoramidites FAM, HEX (MWG Biotech, Courtaboeuf, France) or NED (Applied Biosystems, Courtaboeuf, France) at the 5' position], and 2 to 8 pmol for unlabeled, 1 U of Taq DNA polymerase (Sigma-Aldrich, Lyon, France).

Amplification reactions were performed in a thermocycler (MJ Research, Waltham, MA). The PCR conditions were as follows: after 5 min denaturation at 94 °C, 35 cycles were performed with 30 s denaturation at 94 °C; 1 min annealing at 55 °C; 1 min elongation at 72 °C; and a final extension step of 7 min elongation at 72 °C. PCR products (2 µl) was mixed with 7.9 formamide and 0.1 µl Gensize 400HD (Rox Size Standard; Applied Biosystems) and were separated using capillary electrophoresis on an ABI prism 3130 XL automatic DNA sequencer (Applied Biosystems).

Electropherograms were then analyzed with the Gene Mapper 3.7 software (Applied Biosystems). Allele peak profiles were detected at each locus and genotype, manually reviewed and final sizes were rounded to the nearest full number representing the final called allele length.

### Data analysis

For each SSR locus, alleles were detected and identified by allele size in base pair (bp) and the number of alleles per locus (A). Different indices of genetic diversity were estimated for each locus: observed heterozygosity (Ho), expected heterozygosity (He), and the Wright inbreeding coefficient (Fis) were computed using the software Genetix 4.5 (Weir and Cockerham, 1984; Belkhir et al., 2004). Polymorphic Information Content (PIC) was calculated using CERVUS 3.03 (Marshall, 1998).

Individuals in relationships were represented by dendrogram constructed from Nei's standard genetic distances (Tamura and Nei, 1993); this dendrogram was generated using Unweighted Pair-Group Method using Arithmetic average (UPGMA) (Saitou and Nei, 1987) and was constructed using the software Clustering Calculator (Brzustowski, 2002). The Factorial Correspondence Analysis (FCA) was also recorded on individual plant using Darwin 3.6 software (Perrier et al., 2003).

## Results

### Simple sequence repeat polymorphism

A total of 64 alleles were amplified and the number of alleles per marker ranged from five to eighteen with an average of 10.66 alleles and amplification fragment sizes between 135 and 294 nucleotides (Table 3).

**Table 3.** Locus name and genetic parameters of the South-Algerian fig trees

| Locus  | N     | Size range | Ho   | He   | Fis    | PIC  |
|--------|-------|------------|------|------|--------|------|
| MFC2   | 10    | 140-166    | 0.59 | 0.76 | 0.171  | 0.74 |
| FSYCO1 | 13    | 135-166    | 0.79 | 0.85 | 0.052  | 0.84 |
| LMFC26 | 6     | 217-242    | 0.36 | 0.62 | 0.272  | 0.58 |
| MFC8   | 18    | 147-206    | 0.47 | 0.88 | 0.433  | 0.88 |
| MFC12  | 5     | 151-174    | 0.82 | 0.54 | -0.538 | 0.45 |
| LMFC30 | 12    | 264-294    | 0.65 | 0.75 | 0.125  | 0.72 |
| Mean   | 10.66 | /          | 0.61 | 0.73 | 0.085  | 0.70 |

N, Number of alleles; Ho, Observed heterozygosity; He, Expected heterozygosity; Fis, Wright inbreeding coefficient; PIC, Polymorphic information content

The highest number of alleles (18) was detected at the MFC8, that corresponds with the highest values of the genetic diversity parameters (He = 0.88, Fis = 0.43, PIC = 0.88), whereas the lowest number (5 alleles) was obtained for the MFC12.

The values for observed (Ho) and expected (He) heterozygosities ranged from 0.36 to 0.82, and from 0.54 to 0.88 respectively, the average genetic diversity (He = 0.74) was higher than (Ho = 0.61); according to the Hardy-Weinberg (HW) equilibrium, heterozygote excess (Ho > He) was observed for the MFC12 locus, whereas a

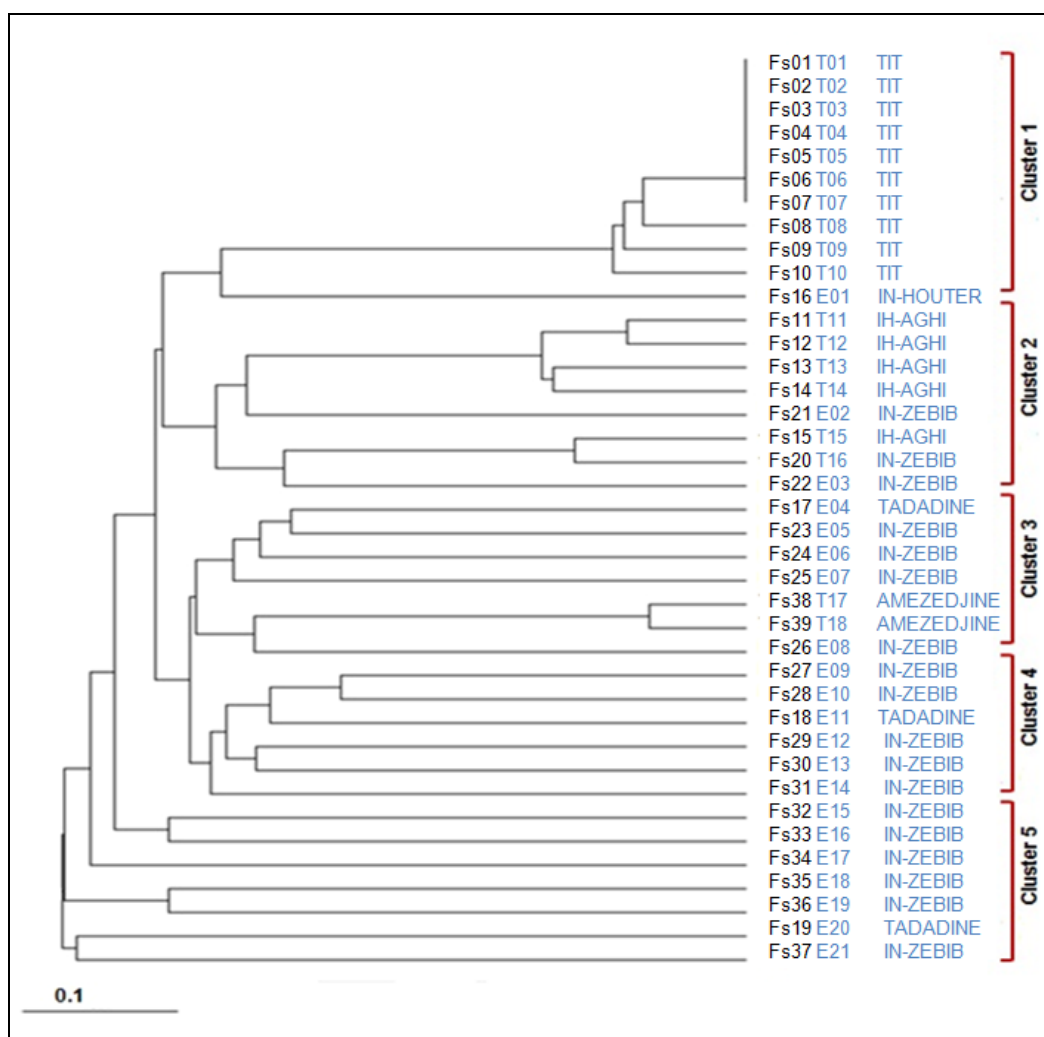
deficiency of heterozygote ( $H_o < H_e$ ) was observed in MFC8, MFC2, LMFC26, LMFC30 and FSYCO1 loci.

The wright inbreeding coefficient ( $F_{is}$ ) values, could be explained by heterozygous deficiency due to positive values of MFC8, MFC2, LMFC26, LMFC30 and FSYCO1 loci, and negative value of MFC12 locus (-0.538), indicating heterozygote excess (Table 3).

The average value of PIC (polymorphic information content) for the primer sets was 0.70, ranging from 0.45 for MFC12 to 0.88 for MFC8. Considering the PIC results, 5 markers (MFC2, MFC8, LMFC26, LMFC30 and FSYCO1) showed PIC values greater than 0.5, therefore, these primers were classified as highly informative and have power for analyzing the genetic variability of *Ficus salicifolia*.

### Genetic relationship between two varieties of *Ficus salicifolia*

To represent the relationships between two varieties of *Ficus salicifolia*, a cluster analysis was used to plot a dendrogram based on Nei's genetic distance (Fig. 3): five main clusters are evident.



**Figure 3.** UPGMA (unweighted pair-group method by arithmetic average) dendrogram based on Nei's genetic distance obtained from analysis of simple sequence repeats data for 39 south Algerian fig trees. *Fs*, *Ficus salicifolia*; *T*, Teloukat; *E*, Eucalyptoides

The first cluster grouped all individuals of *Ficus salicifolia*, variety Teloukat located at Tit (Fs01, Fs02, Fs03, Fs04, Fs05, Fs06, Fs07, Fs08, Fs09, Fs10), only one individual of the variety Eucalyptoïdes (Fs16), located at In-Houter. The trees Fs01 to Fs07 have the same genotype, possibly linked to vegetative propagation.

The second cluster consisting of all individuals of the variety Teloukat located at Ih-Aghi (Fs11, Fs12, Fs13, Fs14, Fs15) and at In-Zebib (Fs20), with 2 individuals of the variety Eucalyptoïdes at In-Zebib (Fs21, Fs22).

The third cluster included the variety Eucalyptoïdes from Tadadine (Fs17) and from In-Zebib (Fs23, Fs24, Fs25, Fs26), and then the variety Teloukat from Amezédjine (Fs38, Fs39).

The fourth cluster formed by all individuals of the variety Eucalyptoïdes located at In-Zebib (Fs27, Fs28, Fs29, Fs30, Fs31) and (Fs18) at Tadadine.

The fifth cluster containing all individuals of the variety Eucalyptoïdes located at In-Zebib (Fs32, Fs33, Fs34, Fs35, Fs36, Fs37) and (Fs19) at Tadadine.

Figure 3 showed that the variety Teloukat was represented by short branches of the dendrogram, but the variety Eucalyptoïdes existed in the long branches.

To elucidate genetic relationships among the 39 fig genotypes, the Factorial Correspondence Analysis (FCA) confirmed information derived from the UPGMA clustering, so, the 2 dimensional scatter plot of factorial correspondence analysis coordinates for the first and second axis, showed a clear separation of the 2 varieties of *Ficus salicifolia* (Teloukat and Eucalyptoïdes) (Fig. 4); however the variety Telloukat was represented by two groups, probably related to the geographical origin of the genotypes.

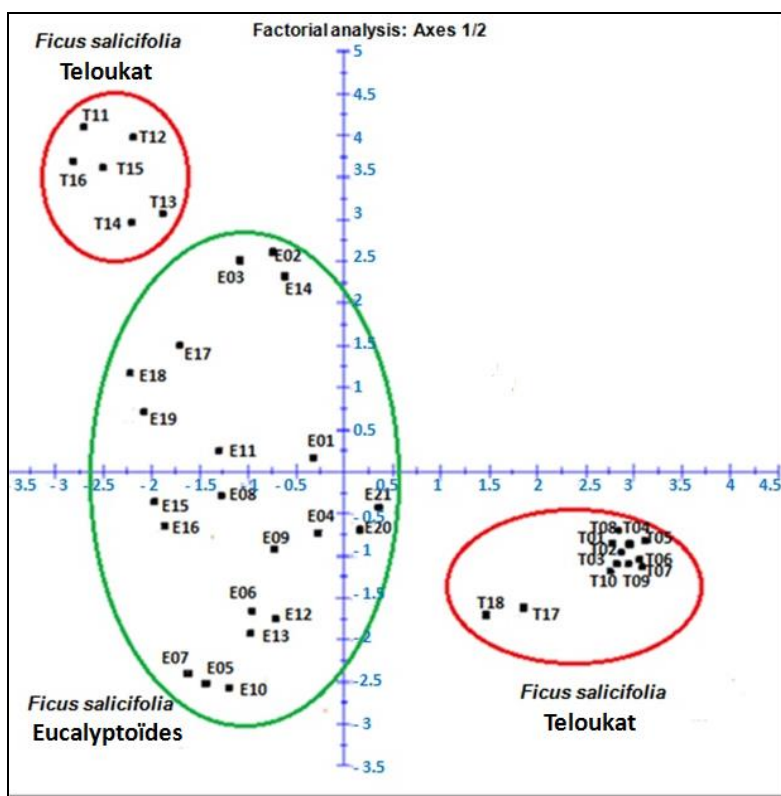


Figure 4. Factorial coordinate analysis (FCA), obtained by SSR markers of 39 south Algerian Fig trees. T, Teloukat; E, Eucalyptoïdes

## Discussion

The microsatellite markers were developed from different species of *Ficus*: *Ficus carica* (Khadari et al., 2001; Giraldo et al., 2005), *Ficus inspida* (Vignes et al., 2006), *Ficus sycomorus* (Ahmed et al., 2007), *Ficus hirta* (Zheng et al., 2015), *Ficus tikoua* (Zhang et al., 2016) and *Ficus virens* (Fu et al., 2017).

The high transferability of microsatellite markers developed from different subgenus of *Ficus* for *Ficus citrifolia* and *Ficus eximia* confirm the general applicability of *Ficus* microsatellite primers to this very large genus (Nazareno et al., 2009). Recently, Ikten et al. (2018), indicated a high level of transferability between *Ficus carica* and related *Ficus* species which provides growing number of SSR for *Ficus carica*; this would have important benefit for species with genomic studies are limited. Moreover, Hladnik et al. (2018) have studied the diversity parameters of selected microsatellites loci for use cultivar identification and fig genetic resources investigations.

In this study, we have made an attempt to use the utility of SSR markers in studying genetic diversity of species of figs, the performance of these markers was evaluated using various parameters such as observed heterozygosity ( $H_o$ ), expected heterozygosity ( $H_e$ ), Wright inbreeding coefficient ( $F_{is}$ ) and Polymorphic information content (PIC).

The SSR loci applied in our analysis detected an average of 10.66 alleles, per locus in 39 individuals fig (*Ficus salicifolia*), which was higher than that found in SSR developed from the *Ficus citrifolia* 7.3 alleles per locus and *Ficus eximia* 6.4 alleles per locus (Nazareno et al., 2009), than from the *Ficus carica*: 5 alleles per locus (Achtak et al., 2009), 4.89 alleles per locus (Ferrara et al., 2016) and 4.2 alleles per locus (Leal et al., 2010). Giraldo et al. (2008) observed a low polymorphism within an ex situ Spanish figs collection, with 3.9 alleles per SSR locus; similarly, Teoman et al. (2017) also found 3.56 alleles per SSR locus within Turkish male and female fig genotypes.

Recently, Boudchicha et al. (2018) published a value of 3.59 alleles per locus within Algerian fig cultivars (*Ficus carica*). The variation in the number of alleles in fig species might be related to the difference in the SSR markers studied, as well as the number of samples and their geographic location (Lopes et al., 2004).

The 6 microsatellites loci used in this work were effective for the characterization of south Algerian fig species with high genetic diversity  $H_o = 0.61$ . Achtak et al. (2009) reported that the average  $H_o$  value was 0.54 for the characterization of Moroccan cultivars figs; however Tunisian germplasm presents a high genetic diversity with  $H_o = 0.62$  (Ben Abdelkrim et al., 2015). Heterozygosity values of *Ficus citrifolia* and *Ficus eximia* reported by Nazareno et al. (2009) were respectively 0.67 and 0.69.

Moreover, the analysis of European and Asian fig cultivars showed a lower level of diversity with  $H_o = 0.44$  (Ikegami et al., 2009), similar results were obtained to characterize a collection of Turkish male and female fig genotypes with  $H_o = 0.45$  (Teoman et al., 2017) and Algerian fig cultivars (*Ficus carica*) with  $H_o = 0.46$  (Boudchicha et al., 2018), similarly, Giraldo et al. (2008) also determined low polymorphism within an ex situ Spanish collection figs with  $H_o = 0.41$ .

According to the Hardy-Weinberg equilibrium (HWE), heterozygote excess ( $H_o > H_e$ ) was observed for the MFC12 locus, and a deficiency of heterozygote ( $H_o < H_e$ ) was observed in MFC2, MFC8, LMFC26, LMFC30 and FSYCO1 loci.

The average of polymorphism information content (PIC = 0.70) is relatively the same compared with that of previous studies (Ikegami et al., 2009; Teoman et al., 2017).



Fis values which is a measure of the deviation of genotypic frequencies from panmixia in population in term of heterozygous deficiency or excess, showed that loss of heterozygosity at just one microsatellite (MFC12). Fixation index values give an idea in terms of the inbreeding coefficient and individual differences.

This finding shows that most of the genetic diversity is caused by the difference between the two varieties of *Ficus salicifolia* as leaf morphology for instance: (the variety Teloukat: the leaves are oblong lanceolate, more or less strung at the base; the variety Eucalyptoides: the leaves are narrowly lanceolate, rounded or sub-attenuated at the base). According to our results, the SSR markers genotyping were in line with the phenotypic identification. The two groups of this variety observed might be linked to the geographical origin of the genotypes (Essid et al., 2015). However, for some groups, the individuals of the same variety were not well separated, probably due to the pollination of this species which is related to the pollinating insect (Jousselin et al., 2003), or to technical errors of the method of analysis used (absence of specific markers).

In addition, in natural habitats, *Ficus salicifolia* has substantially regressed with effects of climate changes; therefore, long term persistence of this taxon requires urgent preservation.

The microsatellite markers used in this study are highly polymorphic and efficient in revealing the level of genetic diversity studied; results from this investigation well demonstrate the capacity of SSR markers to separate genotype on 2 varieties from *Ficus salicifolia* (Teloukat and Eucalyptoides).

## Conclusion

This study reports for the first time the characterization of two varieties of *Ficus salicifolia*, endangered fig species of southern Algeria. A high intra-specific polymorphism recorded by the microsatellite markers suggests their use to study the genetic diversity and the relationship between the different varieties and species of fig trees. This technique offers the ability to detect extensive polymorphisms by its simplicity and rapidity; furthermore, it is useful, to explore the genetic diversity of this important tree using other molecular markers.

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## APPENDIX

### Appendix I. List of individual fig trees with GPS information

| Local name of collection zones | Number of trees | Code of trees | Code of varieties | Altitude (m) | Latitude  | Longitude |
|--------------------------------|-----------------|---------------|-------------------|--------------|-----------|-----------|
| TIT                            | 10              | Fs01          | T01               | 1086         | 22°57'54" | 05°09'73" |
|                                |                 | Fs02          | T02               | 1085         | 22°57'54" | 05°09'73" |
|                                |                 | Fs03          | T03               | 1086         | 22°57'54" | 05°09'73" |
|                                |                 | Fs04          | T04               | 1081         | 22°57'54" | 05°09'73" |
|                                |                 | Fs05          | T05               | 1084         | 22°57'54" | 05°09'72" |
|                                |                 | Fs06          | T06               | 1081         | 22°57'55" | 05°09'72" |
|                                |                 | Fs07          | T07               | 1084         | 22°57'38" | 05°09'81" |
|                                |                 | Fs08          | T08               | 1081         | 22°57'38" | 05°09'81" |
|                                |                 | Fs09          | T09               | 1088         | 22°57'55" | 05°09'73" |
|                                |                 | Fs10          | T10               | 1082         | 22°57'55" | 05°09'73" |
| IH-AGHI                        | 05              | Fs11          | T11               | 1093         | 23°04'10" | 05°12'85" |
|                                |                 | Fs12          | T12               | 1095         | 23°04'10" | 05°12'84" |
|                                |                 | Fs13          | T13               | 1094         | 23°04'10" | 05°12'84" |
|                                |                 | Fs14          | T14               | 1093         | 23°04'10" | 05°12'84" |
|                                |                 | Fs15          | T15               | 1094         | 23°04'10" | 05°12'85" |
| IN-HOUTER                      | 01              | Fs16          | E01               | 1455         | 23°04'73" | 05°22'85" |

|            |    |      |     |      |           |           |
|------------|----|------|-----|------|-----------|-----------|
| TADADINE   | 03 | Fs17 | E04 | 1315 | 22°49'42" | 05°57'33" |
|            |    | Fs18 | E11 | 1330 | 22°49'42" | 05°57'32" |
|            |    | Fs19 | E20 | 1350 | 22°49'43" | 05°57'33" |
| IN-ZEBIB   | 18 | Fs20 | T16 | 1431 | 22°48'17" | 05°37'22" |
|            |    | Fs21 | E02 | 1425 | 22°48'36" | 05°37'13" |
|            |    | Fs22 | E03 | 1435 | 22°48'16" | 05°37'20" |
|            |    | Fs23 | E05 | 1441 | 22°48'42" | 05°37'27" |
|            |    | Fs24 | E06 | 1456 | 22°48'43" | 05°37'26" |
|            |    | Fs25 | E07 | 1449 | 22°48'57" | 05°37'04" |
|            |    | Fs26 | E08 | 1433 | 22°48'28" | 05°37'34" |
|            |    | Fs27 | E09 | 1428 | 22°48'42" | 05°37'22" |
|            |    | Fs28 | E10 | 1451 | 22°48'43" | 05°37'28" |
|            |    | Fs29 | E12 | 1446 | 22°48'41" | 05°37'89" |
|            |    | Fs30 | E13 | 1459 | 22°48'43" | 05°37'27" |
|            |    | Fs31 | E14 | 1428 | 22°48'42" | 05°37'22" |
|            |    | Fs32 | E15 | 1454 | 22°48'43" | 05°37'24" |
|            |    | Fs33 | E16 | 1452 | 22°48'43" | 05°37'26" |
|            |    | Fs34 | E17 | 1458 | 22°48'44" | 05°37'31" |
|            |    | Fs35 | E18 | 1440 | 22°48'35" | 05°37'61" |
|            |    | Fs36 | E19 | 1448 | 22°48'57" | 05°37'04" |
|            |    | Fs37 | E21 | 1447 | 22°48'26" | 05°37'94" |
| AMEZEDJINE | 02 | Fs38 | T17 | 1162 | 22°36'96" | 05°24'46" |
|            |    | Fs39 | T18 | 1165 | 22°36'96" | 05°24'46" |

Fs, *Ficus salicifolia*, T, Teloukat; E, Eucalyptoides