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# Genetic diversity in Tunisian perennial forage grasses revealed by inter-simple sequence repeats markers

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ABSTRACT

The analysis of genetic variation within and among eight populations of perennial species *Festuca arundinacea* and *Lolium perenne* was based on 199 polymorphic inter-simple sequence repeat markers of which 49 (24.6%) were specific to perennial ryegrass, and 31 (15.5%) discriminate tall fescue. Shannon's index and AMOVA partition revealed that in each species the most ISSR variation occurred between populations, rather than within populations. The analysis of the genetic structure of populations showed a significant differentiation of these two species. The Bayesian analysis isolated each tall fescue population, whereas perennial ryegrass individuals were divided into two groups. The observed clustering was independent of the geographical origin of populations and climatic conditions, supporting a high adaptive power aptitude to large scale of ecological variations. Close molecular homology between cultivars and wild populations suggested a substantial gene flow.



## 1. Introduction

The genera *Lolium and Festuca* (*Poaceae* family) are widely used as a major source of forage in temperate grasslands. They are considered to be very closely related based on morphological, biochemical, and genetic data (Wiesner et al., 1995). They are assumed to have a monophyletic origin, and can hybridize in nature (Elazreg et al., 2011a; Lewis, 1975). *Lolium* species provide high forage quality, but generally lack the persistence and stress tolerance to be found in *Festuca* species (Skibinska et al., 2002). The diploid perennial ryegrass (*Lolium perenne* L.) ($2n = 14$) and the hexaploid tall fescue (*Festuca arundinacea* Schreb.) ($2n = 6x = 42$) species are characterized by an allogamous mating system (Sleper, 1985).

In Northern Tunisia, both species constitute an important cool-season bunch grass, widely used in pasture, lawns, and hays. Currently, the local genetic resources are seriously threatened by genetic erosion as a result of irregular rainfall, overgrazing, reduction of rangeland, and neglected forage exploitations (Chtourou-Ghorbel et al., 2011).

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The inter-simple sequence repeats (ISSR) profiling has been successfully used to DNA fingerprint a wide range of crops in various fields of plant improvement and to discriminate between closely related genotypes (Zietekiewicz et al., 1994; Yao et al., 2008).

In this study, the ISSR technology was applied to generate markers as tools to assess the genetic diversity between and within Tunisian tall fescue and perennial ryegrass populations selected from different geographical sites and bioclimatic origins. The objectives of this study are to (i) evaluate the genetic diversity within and among wild populations and cultivars of both species, (ii) clarify the phylogenetic relationships of these two grass species in order to characterize the local genetic resources.

## 2. Material and methods

### 2.1. Plant material and DNA extraction

Six natural populations of *F. arundinacea* and *L. perenne* collected from different geographic sites of North and North West of Tunisia have been considered. In addition, two cultivars were also analyzed in this study: a local variety of tall fescue, "Mornag", and an introduced cultivar of perennial ryegrass, "Igor", originating from Italy and provided by The General Directorate of Protection and Quality Control of Agricultural Products of The Tunisian Ministry of Agriculture. Ten plants were analyzed for each accession. All considered populations and their pedo-climatic parameters are shown in Table 1.

Total cellular DNA was isolated from individual fresh leaf tissue (Dellaporta et al., 1983). The DNA concentration was assessed spectrophotometrically at 260 nm, and the quality was assessed by the 260/280 ratio and by Ethidium bromide coloration after electrophoresis on 0.8% agarose gel (Sambrook et al., 1989).

### 2.2. Primer screening and PCR-ISSR assays

Seven oligonucleotides complementary to simple sequence repeats based on di-, tri- or tetra-nucleotide repeats were tested. These consisted of six 3'-anchored primers and two non-anchored primers.

Amplification was performed in a total volume of 25 μl including 30 ng of total cellular DNA, 200 μM of each dNTP (DNA polymerization mix; Pharmacia, France), 1.5 mM $MgCl_2$ (Biomatik, France), 60 pg of primers, 2.5 μl of 10 × Taq DNA polymerase buffer, and 1.5 U of Taq DNA polymerase (Biomatik, France). PCR amplifications performed in a Perkin 9700 thermocycler (QBIOgene, France) consisted of 5 min at 94 °C, followed by 35 cycles of 30 s at 94 °C, 90 s at each primer appropriate melting temperature (Tm) and 90 s at 72 °C, followed by a 5 min extension at 72 °C. The PCR-ISSR products were separated by electrophoresis on a 1.5% agarose gel in 0.5 × Tris–borate–EDTA (pH 8.3) buffer for 3 h at 80 V. The amplified fragments were visualized by an ethidium bromide staining method under ultraviolet light (Sambrook et al., 1989).

### 2.3. Data analysis

The polymorphic pattern of ISSR was scored as either present (1) or absent (0) to compile a binary matrix. The ability of the most informative primers to differentiate between accessions was assessed by calculating the percentage of polymorphic bands and estimating their resolving power (Rp) according to the Gilbert et al. formula (Gilbert et al., 1999).

Shannon's index was estimated for each ISSR oligonucleotide to perform intra-population diversity ($H_0$), the average diversity over all populations ($H_{pop}$), the species diversity ($H_{sp}$), the component of diversity within populations ($H_{pop}/H_{sp}$), and the component among populations ($G_{st} = (H_{sp} - H_{pop})/H_{sp}$) (Lewontin, 1972; Bussell, 1999).

An analysis of molecular variance (AMOVA) procedure (Excoffier et al., 1992) was assessed to examine the genetic structure and the partitioning of genetic variation among individuals within populations, among populations within species and between species using ARLEQUIN version 3.1 (Schneider et al., 2000). The gene flow parameter (Nm) was estimated using POPGENE version 1.32 (Yeh et al., 1999). Principal Component Analysis (PCA) was assessed using the SPSS Statistics (SPSS for

**Table 1**
Description of pedo-climatic parameters at the collection sites of *Festuca arundinacea* and *Lolium perenne* populations.

| Species | Population Site | Population code | Latitude (°N) | Longitude (°E) | Pedo-climatic factors | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | Altitude (m) | Rainfall (mm) | Max T. °C | Min T.°C | Soil pH | Soil texture | Anthropogenic disturbances |
| *Festuca arundinacea* | Dogga | FDo | 36° 25′ | 9° 12′ | 510 | 525 | 35.2 | 5.1 | 8.75 | clay | grazed |
| | Tabarka | FTb | 36° 56′ | 8° 47′ | 12 | 1029 | 30.2 | 8.0 | 6.5 | loam | settlement |
| | Sedjnane | FSd | 37°03′ | 9°12′ | 116 | 650 | 32.5 | 7.7 | 6.75 | clay | grazed |
| | Mornag | FCm | 36° 50′ | 10° 11′ | 123 | 425 | 33.1 | 5.7 | 9.25 | clay | cultivated |
| *Lolium perenne* | Menzel Temime | LMT | 36° 49′ | 10° 58′ | 18 | 478 | 29.5 | 8.6 | 8.5 | clay | Grazed |
| | Ain Melliti | LAM | 36° 29′ | 9° 9′ | 580 | 523 | 33.4 | 4.9 | 9.0 | clay | Logged/cleared |
| | Sedjnane | LSd | 37°03′ | 9°12′ | 99 | 906.2 | 32.5 | 7.7 | 8.5 | clay | Fire |
| | Igor | LCI | Introduced cultivar (Italy) | | | | | | | | |