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**TAXONOMIC RELATIONSHIPS AMONG  
SOME REGIONAL SPECIES OF THE GENUS  
*LOLIUM* L. BASED ON MORPHOLOGICAL  
AND MOLECULAR MARKERS**

By

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## Summary and Conclusion

The genus *Lolium* L. consists of eight diploid and tetraploid recognized species and belongs to family Poaceae which is one of the largest families, subfamily Pooideae and tribe Poeae; its distribution in the temperate regions from central Asia, through the Mediterranean to the north-west coast of Europe. It contains a wide range of annual, biennial and perennial species which are commonly used as herbage plants, its species have various forms that differ from each other and are greatly economically important.

The genus provides useful material for genetic diversity and relationships studies. Molecular markers are a fast, highly informative and useful tool used in assessment of population analysis to determine the amount of genetic diversity among the species and among populations within each species then assess the relationships among them. In this study, Inter Simple Sequence Repeat (ISSR) DNA marker was applied for its plenty of advantages in generating high polymorphism among closely related taxa.

The present study intends to assess the genetic diversity and taxonomic relationships among the studied species that represent the Mediterranean basin and Middle East region, by using morphological markers and ISSR molecular marker and determines the relationships within each species as well through providing a successful numerical analysis of the relationships among and within the studied species.

This study deals with 89 accessions representing 40 populations of five species (*Lolium perenne*, *Lolium multiflorum*, *Lolium rigidum*, *Lolium temulentum* and *Lolium persicum*) distributed over 14 Mediterranean and Middle Eastern countries (Morocco, Algeria, Tunisia, Libya, Egypt, Palestine, Jordan, Iraq, Iran, Turkey, Cyprus, Romania, Greece and Albania) distributed over three continents (Africa, Asia and Europe). The study was

depended on 80 qualitative and quantitative morphological characters with 172 character states, proposed by the author in addition to other characters that were discriminative by several authors from Linnaeus (1753) to Boulos (2009). The genetic polymorphism and gene diversity was assessed using five polymorphic ISSR primers in an attempt to solve the complicated problems of the taxonomic status of the collected genotypes.

**The conclusion of the present study depended on the information resulted from:**

1. An artificial dichotomous key based on morphological data.
2. Two-dimensional plots that obtained by principal component analysis (PCA) correlation according to Pearson (1901) based on both morphological and molecular data respectively.
3. Average linkage agglomerative dendrogram based on morphological data according to Schütze and Silverstein (1997), based on the distance among and between the studied species and their populations.
4. Computations that calculated by using PopGene version 1.32 of Yeh *et al.* (2000) based on molecular data, the computations mirrored polymorphism percentage, genetic diversity and its index, gene flow and other important related information.
5. Dendrogram based on Nei's (1978) Genetic distance: Method = UPGMA modified from NEIGHBOR procedure of PHYLIP Version 3.5.

**From the present study it was concluded that:**

1. *Lolium perenne* is differentiated by perennial longevity and unbranched culm; *L. multiflorum* by apex of two-toothed lemma and strong overlapping of auricles; *L. rigidum* by presence of concavities on rachillae, wing-

like glume margins and pointed central nerve and obtuse lemma apex; *L. temulentum* by obtuse ligules, upper glume awned, spike lax, spikelet swollen, lemma very turgid, caryopsis swollen and surface of palea is finely scabrid and *L. persicum* by very long caryopses (reach to 9 mm long).

2. The studied taxa are separated into five independent species according to the PCA plot and the clustering method based on morphological and molecular data.
3. *Lolium perenne*, *L. multiflorum* and *L. rigidum* associated with each other; while *L. persicum* and *L. temulentum* are closely associated with each other, based on morphological and molecular markers; on the other hand, *L. perenne* is more associated to *L. multiflorum* rather than to *L. rigidum* based on the morphological data; while *L. perenne* is more related to *L. rigidum* rather than to *L. multiflorum* based on the molecular data.
4. Morphologically, the relationships among the populations within each species are controlled by their similarities that may be dependent or independent on the geographical distribution.
  4. 1. The populations that present dependently are:
    - a. Within *L. perenne*, populations from Morocco and Iran; populations from Algeria and Romania and populations from Libya and Egypt.
    - b. Within *L. multiflorum*, populations from Morocco, Romania and Iran; populations from Libya and Greece and populations from Egypt, Palestine and Turkey.
    - c. Within *L. rigidum*, populations from Iran, Iraq and Jordan, within them populations from Iraq and Jordan are closely related to each other and populations from Turkey and Cyprus.

- d. Within *L. temulentum*, populations from Libya, Turkey and Albania and populations from Egypt and Greece.
  - e. Within *L. persicum*, populations from Iran and Turkey.
4. 2. Morphologically, the populations that present independently are:
    - a. Within *L. perenne*, population from Cyprus and population from Iraq.
    - b. Within *L. rigidum*, population from Morocco and population from Egypt.
    - c. Within *L. persicum*, population from Libya.
5. There is a high degree of diversity among and within the studied species, represented by high percentage of polymorphism of 95.62% for all populations.
  6. Population of *L. perenne* from Cyprus, *L. rigidum* from Libya, *L. temulentum* from Iraq, *L. multiflorum* from Turkey and *L. persicum* from Turkey are of the highest degrees of gene diversity.
  7. It is predicted that Turkey may be the center of origin of the studied *Lolium* species.
  8. *Lolium multiflorum* from Turkey not only of highest degree of gene diversity, but also of highest degrees of Shannon's information index, observed number of alleles and effective number of alleles.
  9. Migration of the species and seeds exchange between the studied populations is limited.
  10. Gene diversity is non-significant and allele frequencies are not equal for all alleles among the populations of (*L. perenne* from Egypt, Palestine and Iraq; *L.*

*multiflorum* from Morocco, Libya, Palestine, Iran and Greece; *L. rigidum* from Jordan; *L. temulentum* from Morocco, Libya, Egypt, Albania and Greece and *L. persicum* from Libya).

11. Gene flow among all the populations is low; there is high degree of complication and independence of the evolutionary line of the studied species and high degree of genetic variation within each species.
12. *Lolium persicum* is the most variable species and the genetic similarities are low among its populations and *L. rigidum* is the least variable species and the genetic similarities are high among its populations.
13. Molecularly, the closely related populations according to their geographical distribution are:
  - a. Populations of *L. temulentum* from Turkey and Albania.
  - b. Populations of *L. multiflorum* from Turkey and Greece.
  - c. Populations of *L. rigidum* from Iraq and Iran.
  - d. Populations of *L. rigidum* from Turkey and Cyprus.
  - e. Populations of *L. perenne* from Iraq and Iran.
  - f. Populations of *L. perenne* from Greece and Cyprus.
14. Genetic identity regardless to the origin of the populations is in populations of:
  - a. *Lolium temulentum* from Iraq and Greece.
  - b. *Lolium perenne* from Tunisia and Albania.
15. Molecularly, the populations that separated independently are:

- a. Populations of *L. persicum* from: Libya, Iran and Turkey.
  - b. Populations of *L. temulentum* from: Libya, Egypt and Morocco.
  - c. Populations of *L. multiflorum* from: Palestine, Romania, Egypt, Iran, Libya and Morocco.
  - d. Populations of *L. rigidum* from: Jordan, Libya, Greece, Egypt, Algeria and Morocco.
  - e. Populations of *L. perenne* from: Egypt, Palestine, Libya, Algeria and Morocco.
16. The origin of the accession of *L. persicum* (GR 5452) collected in 1983, from Libya that was provided by IPK is doubtful and needs to be revised in its collection in IPK for future studies and to enable its study for other researchers.
17. In the current work, application of the morphological and molecular markers on the studied species and consideration of their numerical treatment is successful and fruitful in detection of genetic diversity, also in assessment of relationships for the studied species and populations; as well as helpful tools in estimation of the species center of origin.