Lentils biodiversity: the characterization of two local landraces

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Abstract — A multi-disciplinary approach was used to characterize two autochthonous lentil landraces from Molise region (Central Italy). Different mature seed populations for each landrace were provided by the Molise Germoplasm Bank at the University of Molise (Pesche, Italy), and analyzed at the morphological and molecular (DNA and protein) levels. Nuclear ISSR markers were used to assess genetic differences, whereas phenotypic variability was detected by biochemical (proteomics) and morphological analyses. The genetic and phenotypic diversity of the two lentil landraces were well assessed in relation to their geographical provenance, supporting further studies to identify landrace markers.

Index Terms — ISSR markers, *Lens culinaris*, seed morphology, proteomics.

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1 INTRODUCTION

Lens culinaris Medik. has been cultivated around the Mediterranean basin since at least the seventh century B.C. and its cultivation area expanded to Middle East, Ethiopia and the Indian Subcontinent ([1], [2]). Local landraces are characterized by high genetic variability and high adaptation to different environmental conditions evolving in adaptive gene complexes [3]. However, in the industrialized countries the cultivation of many different local landraces has progressively decreased, becoming at high risk of genetic erosion ([4], [5]). In this paper, the diversity of two lentil landraces is analyzed to define and quantify differences between groups of populations coming from two geographical areas of Molise region (Central Italy). In particular, this study aims

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to deepen the knowledge about morphological, genetic and proteomic markers that differentiate local lentil landraces in relation to their provenance.

2 MATERIAL AND METHODS

Twelve lentil seed populations from Molise region (Central Italy) were studied in relation to their provenance: five from Conca Casale and seven from Capracotta. The total sample was analyzed by a multi-disciplinary approach, useful to characterize the phenotypical and genotypical traits of the two landraces. For the genetic analysis, nuclear ISSR markers were used as described in a previous paper [3], whereas the biochemical investigation was carried out on total seed proteins extracted according to the method of Rabilloud and resolved by 2DE [3]. For each population, gels were run in triplicate, and the mean value computed by software-assisted (PD-Quest) analysis was used to obtain a standardized matrix of the abundance (relative volume) of protein spots. One hundred seeds of each landrace population were used to measure eight morphological traits: area, perimeter, major axis length, minor axis length, and roundness, 100-seed weight, 100-seed volume and density (g/ml).

2.1 STATISTICAL ANALYSIS

For molecular data, Principal Component Analysis was computed on Nei's genetic distance (1972) and the hierarchical partition of genetic variation among and within populations was obtained by means of the analysis of molecular variance (AMOVA). For biochemical and morphological data, a standardized matrix was subjected to univariate (ANOVA) and multivariate statistical analysis. Principal Component Analysis was computed on significant variables (detected by ANOVA) and the extracted Principal Components (eigenvalues > 1) were used in Canonical Variate Analysis.

3 RESULTS

The results of ISSR analysis pointed out the genetic relationship between the two landraces. As shown in Fig. 1, the PCA highlighted a clear separation of the populations sampled in Conca Casale and Capracotta. In particular, along the first two PCs the total variance accounted for 45.57% and 18.04%, respectively. Molecular degree of differentiation between the two groups of populations (AMOVA) showed a significant molecular discrimination (PhiPT = 0.438; p = 0.001). Moreover, it resulted that the genetic variability was greater within (56%) than among (44%) groups of landrace populations.

The comparison of total seed proteomic maps of the Conca Casale and Capracotta populations revealed a total of 193 differentially expressed proteins. The biochemical data set (193 proteins) was subjected to ANOVA, to identify biochemical markers useful to distinguish lentil populations from different provenances. It resulted that 25 proteins were significant to discriminate Capracotta from Conca Casale lentils. PCA was computed on a correlation matrix, using these 25 significant proteins; the first two PCs explained 53.79%

and 11.62% of total variance, respectively, and the scatter plot of these two PCs indicated a clear distinction between the two groups of lentils (Fig. 2). Differences between the two landraces were tested by canonical variate analysis (CVA) computed on the extracted PCs. They were significantly discriminated (Wilks' $\lambda = 0.028$; df=5; p< 0.0001) as shown by the test of cross-validation (100% of cases were correctly classified).

The eight morphological variables were subjected to ANOVA in relation to population provenance. The two groups of lentil populations from Capracotta and Conca Casale were significantly discriminated by six morphological traits: seed density, roundness, volume, major axis length, perimeter and minor axis length. These six variables were used to compute a PCA: respectively, PC1 and PC2 explained 80.45% and 18.30% of total variance, highlighting a clear separation between landraces (Fig. 3). Then, the PCs were used in CVA and results indicated significant differences between the two population groups (Wilks' $\lambda = 0.047$; df = 2; sig.< 0.0001). Moreover, the test of cross-validations showed a high significance of the CVA reporting that 100% of cases was correctly classified.
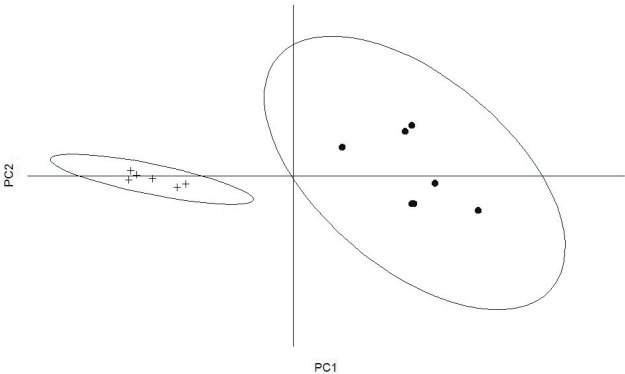

Fig. 1 – Scatter plot of specimens (cross = Conca Casale; point = Capracotta) ordered along the first two principal components; PCA from molecular data.
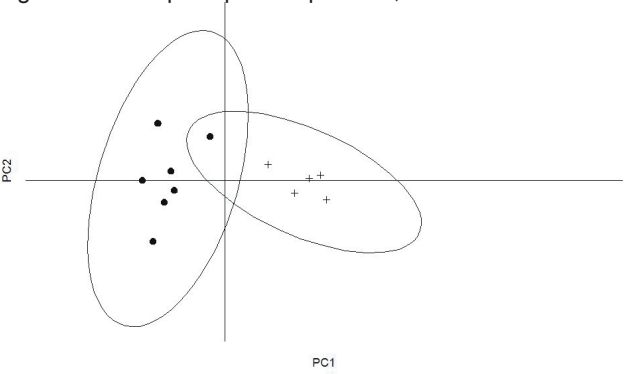

Fig. 2 – Scatter plot of specimens (cross = Conca Casale; point = Capracotta) ordered along the first two principal components; PCA computed on 25 proteins.
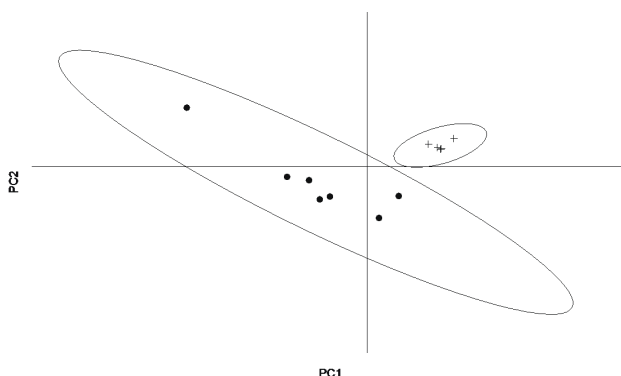
Fig. 3 – Scatter plot of specimens (cross = Conca Casale; point = Capracotta) ordered along the first two principal components; PCA computed on six morphological variables.

4 CONCLUSION

Autochthonous plant germplams, characterized by a wide genetic variability and high adaptation to different environmental conditions, are often more subjected to genetic erosion risks. In Italy, several different lentil landraces evolved thanks to the combination of different geographical characteristics.

The literature reports a wide variety of methods that have been used to investigate genetic similarities and relations among landraces of *L. culinaris* Medik.

Different methods have different powers of genetic resolution and provide different information: neutral DNA markers are useful tools to describe genetic relations in terms of time divergence [6], whereas phenotypic markers can provide information about adaptive responses to macro-environmental conditions [7].

In this study we used a combination of genetic and phenotypic analyses to characterize two autochthonous lentil landraces of two different provenances within a small region such as Molise.

The integration of genetic markers analysis with seed morphology and proteomic traits provided a high resolution approach to dissect lentil biodiversity [3]. The diversity between groups of populations, coming from two very close geographical areas, was well assessed and quantified. In addition, differences between the two local landraces were principally related to their sites of origin, where climate conditions and human activity may have selected the local accessions characterised by specific morphological and biochemical traits of seeds. Work is in progress to deepen the relation between these phenotypic markers and the environmental characteristics of the landrace provenance areas, and to identify the seed proteome markers.

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