

## GENOMIC AND PROTEOMIC ANALYSES OF ZEINS IN INBRED LINES AND LOMBARD VARIETIES OF MAIZE

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DNA barcoding, the use of a short standardised region of DNA for identifying either species and races, has been used successfully in animal field since 2003. However, optimisation of botanical barcoding has been more challenging. Although numerous strategies have been proposed, determination of the right stretch of plant DNA has been difficult.

In this study we exploited the possible use of *zein* genes for a rapid identification of maize lines and varieties. We choose *zeins* because they are a multigenic family (about 100 genes per haploid genome) that shows an extreme variability at the genic level and, consequently, a complex heterogeneity at protein level.

Zeins are storage ethanol-soluble proteins accumulated during seed development and they account for 50–70% of the total endosperm proteins of maize (*Zea mays*) and its wild ancestor *Teosinte*. Based on their solubility, their genetic properties and their apparent molecular masses, zeins have been classified into  $\alpha$ - (22 and 19 kDa),  $\beta$ - (14 kDa),  $\gamma$ - (27 and 16 kDa) and  $\delta$ - (10 kDa) zeins. The complexity level of peptide components in the zein fraction can be clearly shown by an analysis that combines the techniques of isoelectric focusing and SDS gel electrophoresis (2D electrophoresis).

We analysed zein fraction from several maize lines: the two well characterized lines at the genomic level (i.e. B73 and Mo17) and the four wild-type maize lines the most frequently used in previous genetic, biochemical and molecular analyses (i.e. BSSS53, W64A, W22, NYR and A69Y) together with some of their isogenic lines carrying mutant alleles affecting zein expression. These lines were used as reference to perform a systematic analysis of several Lombard varieties both at proteomic and genomic level of zein constituents. We obtain for each variety a distinctive 2D profile characterized by numerous isoforms within the molecular mass classes and charge for all zeins. These data constitute the *zein proteome profile* (zPP) that reveals a unique pattern for each variety. This indicates a high heterogeneity in the coding sequence most probably based on SNPs leading to amino acid substitutions of charged or polar residues. Sequence analyses of more than 150 zein genes confirmed the above hypothesis. At genomic level each maize line or Lombard variety manifests a unique RFLP pattern, *zein genome profile* (zGP).

Based on these results a specific set of primers has been developed for direct analysis by PCR multiplex methodology.