Proceedings of the 52nd Italian Society of Agricultural Genetics Annual Congress
Padova, Italy – 14/17 September, 2008
ISBN **978-88-900622-8-5**

Poster Abstract – A.02

DETECTION AND QUANTIFICATION OF DIFFERENT POLLEN TAXA BY MEANS OF A REAL-TIME PCR APPROACH

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pollen detection, pollen quantification, Real Time PCR, allergy

A specific, sensitive real-time PCR assay based on TaqMan chemistry was developed for the identification and quantification of air dispersed pollen, in order to provide a rapid and reliable analysis alternative to the traditional and time-consuming microscope based detection methods. Actually, accurate and punctual information of the amount and type of air-dispersed pollen represents a useful tool for the diagnosis and therapy of allergy, moreover it can be essential for monitoring the pollen diffusion of GMOs.

A set of ten pollen taxa was considered for their presence in the local flora and their allergic potential. *Parietaria officinalis*, *Ostrya carpinifolia* and species belonging to *Alnus*, *Betula*, *Artemisia*, *Corylus*, *Fraxinus* genus and *Poaceae* (*Gramineae*) and *Cupressaceae* families were finally tested. For each taxon, pollen and leaves were sampled in three different areas of Trentino region, in order to represent genetic differences within different plant populations.

Bioinformatic analysis was carried out to identify specific DNA sequences for each plant group. The search at NCBI database was focused on single- or low-copy nuclear gene sequences. Performing a BLAST against the non redundant nucleotide database, the specificity of DNA sequences was assessed *in silico*. Amplification of target genomic regions with degenerate primers and sequencing of PCR products were performed when no informative DNA sequences were available such for *Parietaria* and *Poaceae*.

In parallel, an efficient protocol for DNA isolation from both free and sampling strip-immobilized pollen was established. After setting the appropriate real-time PCR conditions, the specificity of primers and probe was verified using DNA isolated either from leaves or pollen.

Specific primer-probe combinations were finally established for the following groups: *Parietaria officinalis, Artemisia annua*, *Betula pendula*, *Alnus, Ostrya carpinifolia, Olea europea*, *Graminacae, Oleaceae* and *Cupressaceae.*

DNA isolated from different pollen species was used for standard curve construction when taxa had to be identified at genus or family level. Preliminary results of quantification analysis of pollen mixtures will be presented.