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Poster Abstract – E.02

DETECTION OF TRANSGENE COPY NUMBER IN DURUM WHEAT TRANSGENIC LINES USING REAL-TIME PCR

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Genetic transformation has played a key role in gaining and applying knowledge of the roles of HMW-GS in wheat end-use properties. Reliable and stable expression of transgenes as well as the characterization and field adaptation of transgenic lines are prerequisites for the successful application of gene technology.

Loci that appear to be stably expressed initially can become progressively silenced over generations. The stability and the behaviour of transgenes are influenced by several factors, such as chromosomal location, transgene copy number and their interaction with the host genotype.

Traditionally such factors are characterized using Southern analysis which can be time consuming and laborious. Recent results obtained in various crops indicate that Real-Time PCR could be a powerful tool for the detection and characterization of transgene locus structures. The determination of transgenic locus number through Real-Time PCR overcomes the problems linked to phenotypic segregation analysis and can analyze hundreds of samples in a day making it an efficient method for estimating copy number integrated in a transgenic line.

This study was conducted to determine transgene copy number in transgenic lines and to investigate potential differences in sensitivity, resolution and variability between two different Real-Time chemistries (SYBR Green dye and TaqMan probes). We have applied Real-Time PCR to a set of four transgenic durum wheat lines previously obtained. A total of six experiments (two experiments for each gene) were conducted and standard curves were obtained from serial dilutions of the plasmids containing the genes of interest. The correlation coefficients of the standard curves were rather good, being in the range between 0.95 and 0.97. By using TaqMan quantitative Real-time PCR we were able to achieve estimates of 1 to 42 copies of transgenes per haploid genome in T_4 homozygous transformants. Conversely, SYBR Green dye method revealed unable to accurately quantify transgene copy number as it failed in detecting the inserted genes when integrated in few copies.

In our study we assessed Real-Time PCR as a fast, sensitive and reliable method for the detection of transgene copies in durum wheat, which can be a valid alternative to Southern analysis.