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Poster Abstract – D.19

IDENTIFICATION AND VALIDATION OF REFERENCE GENES FOR QUANTITATIVE RT-PCR NORMALIZATION IN WHEAT

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Reference genes with highly uniform expression across different tissues, developmental phases, environments and experimental treatments are required both in advanced (microarray and real time PCR) and traditional (northern) methods of gene expression evaluation and transcript quantification. In plants the search of new reference genes is very limited, whereas most studies concern the evaluation, comparison and validation of well-known reference genes in different species and experimental conditions. Usually the reference genes have been chosen for their known or suspected housekeeping roles in basic cellular processes, such as cell structure maintenance or primary metabolism. However, the variation observed in transcription levels of most housekeeping genes hinders their effective use in gene expression analyses. Cultivated bread wheat (*Triticum aestivum* L.) is an allohexaploid species (AABBDD) with three very large homoeologous genomes, each comprising seven pairs of chromosomes. Since the wheat genome is too large to be entirely sequenced in the near future, structural and functional analysis of the wheat transcriptome is particularly important. The screening of the whole wheat transcriptome has recently been made possible by the availability of an Affymetrix GeneChip Wheat Genome Array containing 61,127 probe sets (55,052 transcripts), likely covering half of the wheat expressed genes. Since qRT-PCR is necessary for array validation and in-depth expression studies, it is important to identify reliable internal control genes suitable for many experimental conditions. Using the terms “wheat”, “gene expression”, and “real time RT-PCR” combined by the Boolean operator “AND” we performed a PubMed search of articles published from January 1996 to March 2008. We had access to 26 articles that used 16 different reference genes. Remarkably, genes encoding 18S rRNA (8 times, 30%), actin (7 times, 27%) and alpha-tubulin (5 times, 19%) have been used in about two third of the studies, whereas other cited reference genes accounted for 4 to 11%. For the normalization of the expression data multiple reference genes have been used in five studies only, whereas in 16 researches single reference genes have been used, presuming their stability of expression without any preliminary validation. The lack of validated reference genes for expression studies in wheat, evidenced by our literature survey, clearly emphasizes the importance of a systematic study for identifying more reliable control genes.

In this study several novel candidate reference genes suitable for gene expression normalization in wheat were identified by a cross search for stable expression in Unigene and TIGR databases. We selected 38 genes representing different functional classes, whose expression was assessed by qRT-PCR in RNAs from 18 wheat tissues and floral organs and in seedlings exposed to low and high temperatures. Additionally, we carried out a comprehensive evaluation of the expression patterns of the actin and alpha-tubulin gene families, which have commonly been used as controls for normalization of gene expression in wheat. Quantitative RT-PCR analyses were

performed by primer pairs designed to target either single genes or two or more members of the same gene family. The expression stability of the genes was estimated by three statistical approaches: 1) cycle threshold (Ct) variation range and coefficient of variation; and 2) geNorm and 3) Norm Finder programs. Finally, we showed that the use of unsuitable reference genes caused a wrong evaluation of relative expression levels of the genes encoding a MADS-box and a PDI-like protein in the analysed tissues.