

DEVELOPMENT OF A NEW WHEAT MICROARRAY FROM A TOTIPOTENT CDNA LIBRARY AND IDENTIFICATION OF DIFFERENTIALLY EXPRESSED GENES FOR POWDERY MILDEW RESISTANCE

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In the present work we reported an innovative method for gene discovery based on the use of suitable quantities of 5-azaCytidine in the germination phase of plants to induce the de-methylation of the total DNA. This led to the obtainment of seedlings potentially expressing whole the genes present in the target genome in an early plant stage avoiding plant regulation systems. On this basis a new wheat microarray consisting of 4925 expressed sequence tags, was developed from a totipotent cDNA library and used for the screening of two near isogenic lines, cv. Latino of durum wheat and the line 5BIL-42 susceptible and resistant respectively, to powdery mildew. In order to identify genes involved in resistance responders, the two isogenic lines were infected and grown in greenhouse under controlled condition. Three replicas for each treatment has been carried out. The seedlings were grown with a temperature of 22°C under continuous light. A single isolate (O2) of powdery mildew was used for inoculation seedlings. Leaves samples were collected after 24, 48 and 72 hours from incubation at 22°C under continuous light in the two isogenic lines and immediately frozen at -80°C for RNA extraction.

The RNA samples were extracted and reverse transcribed into cDNA, labelled using two different fluorophores (Cy3, Cy5) and then hybridised simultaneously to each glass slide. Six genes were found differently expressed in two isogenic lines. Search similarity of these sequences in public databases indicated for four sequences a putative function while two were unknown. Physical mapping of the six genes located the accession AJ611689 on chromosome 5BL in the centromeric bin where the resistant *Pm36* gene was previously located, indicating this as the putative resistance gene.