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Poster Abstract - B.28

FISH-BASED PHYSICAL MAPPING OF A LOW-MOLECULAR-WEIGHT GLUTENIN SUBUNIT TRANSGENE INSERTION IN A BREAD WHEAT LINE OVER-EXPRESSING THE TRANSGENIC SUBUNIT

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Fluorescence in situ hybridization (FISH) on somatic metaphase chromosomes was used to determine the physical location of a low-molecular-weight glutenin subunit (LMW-GS) gene, which was introduced by microprojectile bombardment into the bread wheat cv. Bobwhite. In the transformed target line (BOB-T), the LMW-GS transgene, driven by its own promoter, was estimated to be 12-16 times over-expressed with respect to endogenous LMW-GS. Based on Southern analysis, this over-expression was attributed to the insertion of multiple transgene copies.

For FISH experiments, a 3.7 kb insert from the pLMWF23A plasmid, used for transformation and containing the coding region of the LMW-GS gene flanked by about 1200 bp and 1600 bp of the 5' and 3' regions, respectively, was employed as a probe. For genome karyotyping, additional probes were used, corresponding to highly repeated DNA sequences predominantly hybridizing to B-genome (pSc119.2) or D-genome (pAs1) chromosomes, and to 18S-5.8S-26S rDNA sites (pTa71). FISH with such probes was carried out on BOB-T line and on control lines, i.e. a "null", non expressor line, isolated by segregation from the original transformed line, and normal Bobwhite.

FISH with multiple probes revealed a single, prominent hybridization site in the BOB-T genome, located in the pericentromeric region of the long arm of chromosome 5D, as evidenced by its specific pAs1 pattern and by the presence of a rDNA site at the 5DS telomere. Judging from its fluorescence intensity, the 5DL site might well include several copies of the transgene and/or portions of it. No hybridization site for the transgene probe was detected in the control lines.

To avoid ambiguous results possibly due to cross-hybridization between vectors' sequences, inserts devoid of such sequences were employed for FISH. However, a false evidence was initially obtained, consisting of the presence of an additional pAs1 site on BOB-T 5DL, nearly coinciding with the transgene hybridization site. Comparison of the transgene and pAs1 sequences revealed considerable identity between the two. A modified FISH protocol, including a preannealing step between the two probes, aimed at subtracting homologous sequences, allowed to ascertain the actual absence of such a chromosomal rearrangement in the vicinity of the transgene locus. As a whole, taking as reference the normal distribution pattern of the highly repeated DNA probes used for genome karyotyping, no cytologically detectable chromosomal rearrangement seemed to be associated with the transgene insertion.

Given the resolution limits inherently associated with contracted metaphase chromosomes, the existence of other transgene loci, containing lower copy numbers than that on 5DL, cannot be excluded. To unequivocally associate the transgene over-expression with the 5DL locus, a monosomic analysis is

currently under way. This should reveal absence of over-expression to be only associated with nullisomic-5D F_2 segregates from the cross between BOB-T and a monosomic-5D line.

Finally, because the BOB-T line was co-transformed with a UBI:BAR plasmid DNA clone, the insertion pattern of the reporter gene plasmid is also being analysed by FISH.