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A MULTIDIMENSIONAL ANALYSIS OF COMPRESSIBLE AND INCOMPRESSIBLE FLOW FLUID DYNAMICS PROTEIN SEPARATION BY POLYACRYLAMIDE GEL ELECTROPHORENSIS

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by

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Brian Steven Brown

4 single spaces

A dissertation submitted to the faculty of
The University of North Carolina at Charlotte
in partial fulfillment of the requirements
for the degree of Doctor of Philosophy in
Applied Mathematics

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Charlotte

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2013

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ISOLATION AND CHARACTERIZATION OF *TRITICUM AESTIVUM* ROOT cDNA SEQUENCES WHICH SHOW SIGNIFICANT HOMOLOGY TO THE GRAMNEGATIVE BACTERIAL TRANSPOSON TN*1721*

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Jennifer Renee Brown

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ABSTRACT

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JENNIFER RENEE BALL. Isolation and characterization of *Triticum aestivum* root cDNA sequences which show significant homology to the gram-negative bacterial transposon Tn*1721*. (Under the direction of DR. HELEN W. JOHNSON)

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While previously screening a bread wheat (*Triticum aestivum* cv. Pavon) pollen embryo cDNA library, four clones were identified that showed high homology to the bacterial transposon Tn*1721*. Finding these clones led to studies to ascertain whether the bacterial sequence was actually present in the wheat genome or was simply an artifact of the cloning procedure. Using the transposon as a probe, a root cDNA library was screened. Two putative clones were isolated from this library and sequenced in both directions. Consensus sequences were used to search the nucleotide databases for homologies. This search revealed that both clones were highly homologous to the *tetR* gene of Tn*1721*. Northern hybridization using RNA isolated from roots confirmed that the Tn*1721* sequence was present in genes expressed by this tissue. These results support the hypothesis that regions of the Tn*1721* transposon are integrated into the wheat chromosomal DNA and that this may be an example of an interkingdom horizontal transfer of DNA from a bacterium to a plant.

ACKNOWLEDGMENTS

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The important element in the Acknowledgments is simple courtesy in which there are usually two possible ingredients to consider. First you should acknowledge any significant help you received from any individual whether in your department or elsewhere. Specifically, you should acknowledge the source of special materials, documents, or equipment. Further, you should acknowledge the help of anyone who contributed significantly to the work or to the interpretation of the work. Second, you should acknowledge any outside source of financial assistance, such as grants, contracts, or fellowships. A word of caution is in order. Often it is wise to show the proposed wording of the Acknowledgments to the person whose help you are acknowledging. He or she might well believe that your acknowledgment is insufficient or (worse) that it is too effusive.

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LIST OF ABBREVIATIONS

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ABA	abscisic acid
ABRE	abscisic acid response element
ANOVA	analysis of variance
BHT	butylated hydroxytoluene
cDNA	complementary DNA
cpm	counts per minute
DS	dextran sulfate
dATP	deoxyATP
ddATP	dideoxyATP
2,4-D	2,4-dichlorophenoxyacetic acid
EcMt	early cysteine-labeled metallothionein
KN	Kinetin
mAb	monoclonal antibody
mRNA	messenger RNA

CHAPTER 1: INTRODUCTION

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Transposons are genetic elements that are mobile within a genome, therefore, they are one of the major causes of genomic variation (Lewin 1994). However, evidence is growing that transposable elements are capable of horizontal transfer. That is, they can move across genomes of different species (Prins and Zadocks 1992). Our laboratory is interested in the identification and characterization of bacterial transposon-like nucleotide sequences found in the wheat genome that may be an example of horizontal DNA transfer.

Reynolds and Kitto (1992) screened a Mexican spring wheat (*Triticum aestivum* cv.Pavon) cDNA library to identify genes expressed specifically during pollen embryogenesis. After sequencing unique clones from this library, four sequences were found that showed high homology to the bacterial transposon, Tn*1721*. This transposon was derived from a gram-negative bacterium and is a Tn*3*-like transposon found in the Tn*21* subgroup (Grinstead et al. 1990). It is a unique sequence since it contains a basic transposon (Tn*1722*) that is capable of independent transpososition. As shown in Figure 1, Tn*1722* contains an open reading frame that encodes a 525 amino acid chemotaxic protein (Allmeier et al. 1992). The Tn*1722* portion of the transposon contains the *tnpR* and *tnpA* genes which are utilized during the genetic resolution and integration of either the major or minor sequences. The entire transposable element also include three inverted repeats which function as the insertion and excisions sites for the transposon.

REFERENCES

2 single spaces, start the next line on the 3rd

Allmeier H, B Cresnar, M Greck, and R Schmidt. 1992. Complete nucleotide sequence of Tn*1721*: gene organization and a novel gene product with the features of a chemotaxic protein. Gene 111: 11-20.

Gierl A and H Saedler. 1992. Plant transposable elements and gene tagging. Plant Molec Biology 19: 39-49.

Herman H, M Jacobs, JB Clark and MN Majerius. 1990. Plant chromosome-marker gene fusion assay for the study of normal and truncated T-DNA insertion events. Molec Gen Genetics 224: 248-252.

Houck MA, D Hurst, MN Majerius. 1992. Selfish genes move sideways. Nature 359: 659-660.

Lewin B. 1994. Genes V. Oxford University Press, Oxford.

Melanson D. 1995. Molecular characterization of nucleotide sequences from bread wheat (*Triticum aestivum*) which show homology to the gram-negative bacterial transposon, Tn*1721*. Master's Thesis, University of North Carolina at Charlotte, Charlotte, North Carolina.

Prins TW and JC Zadoks. 1994. Horizontal gene transfer in plants, a biohazard? Euphytica 76: 133-138.

Reynolds, TL. 1993. A cytological analysis of microspores of *Triticum aestivum* (Poaceae) during normal ontogeny and induced embryogenic development. Plant Physiol 80: 596-605.

Reynolds, TL. 1994. Vector DNA artifacts in the nucleotide sequence database. BioTechniques 16: 1124-1125.

Schmitt R, E Bernhardt, and R. Mattes. 1996. Characterization of Tn*1721*, a new transposon containing the tetracycline resistance genes capable of amplification. Molec Gen Genetics 172: 53-65.

Sigee DC. 1993. Bacterial plant pathology: the myth and the legends. Cambridge University Press, London.

Tienes RF, GH Waggnor, LJ Phillips, and YMN Bajaj. 1995. Are transposons capable of horizontal movement between species? Molec Biology 56: 1025-1034.

APPENDIX A: PCR OPTIMIZATION

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The optimization of the PCR reactions were conducted on the bacterial plasmid pJOE105, which contains the entire Tn*1721* transposon. This optimization required three independent experiments to determine the best parameters for each set of primers: $MgCl_2$ concentration, target DNA concentration, and thermal cycling parameters.

Magnesium provides the divalent cations required by the DNA polymerase to function. The $MgCl_2$ concentrations were optimized by titration reactions ranging from 1.55 mM to 3.55 mM final concentration in each reaction tube.

The concentration of target DNA was optimized to ensure the highest possible primer specificity. DNA was diluted serially for each reaction to determine the lowest concentration of polynucleotide that still yielded visible bands on EtBr-stained agarose gels; for pJOE105 this was # 1 ng of DNA.

PCR cycle parameters were examined to reduce the so called plateau effect which results in the non-specific amplification of background products. Taking this into account, cycling parameters were set to allow efficient amplification with the lowest number of cycles. Conditions were set at 33 cycles of 1 min. at 94 C for denaturation, 1 min. at 56.5 C for annealing, and 2 min. at 72 C for synthesis, followed by 10 min. at 72 C for extension.