Toxicity of Wheat Non-Specific Lipid Transfer Proteins to *Tilletia tritici*, the Common Bunt Pathogen

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Abstract: Non-specific-Lipid Transfer Proteins (ns-LTPs) have the potential to exhibit both lipid transfer activity and antimicrobial activity. Eleven novel full-length wheat ns-LTP1 clones, all possessing coding sequences of 348 bp, isolated from abiotic- and bunt-stressed cDNA libraries, exhibited highly conserved coding regions with 78–99% and 71–100% identity at the nucleotide and amino acid levels, respectively. The deduced proteins coded by these cDNAs were characteristic of the ns-LTP1 family. The full-length wheat ns-LTP1 genes were cloned into the expression vector pPICZα and transformed into *Pichia* yeast. SDS-PAGE, Western blotting and *in vitro* lipid binding activity assay confirmed that the eight ns-LTPs were all successfully expressed and capable of *in vitro* binding of fatty acids. A large scale comparative study of the toxicity of eight wheat ns-LTPs to growth of mycelium or spores of eight wheat pathogens including *T. tritici*, and three non-wheat pathogens demonstrated a differential toxicity among the ns-LTPs. LD_{50} values of three selected ns-LTPs against six fungi ranged from 1 to 7 μmol/l. *In vitro* lipid binding activity of ns-LTPs was not correlated with their antifungal activity. Using the fluorescent probe SYTOX Green as an indicator of membrane integrity, the toxicity of wheat ns-LTPs was shown to be associated with an alteration in fungal membrane permeability.

Keywords: antifungal activity; ns-LPTs; wheat pathogens; membrane integrity