

Generation of Recombinant Antibodies against Orchardgrass Acidic nsLTP-Like Proteins

Gorica Rakleova, Ivanka Tsacheva, Mima Petkova, Ivelyn Pantchev, and Magdalena Tchorbadjieva*

Department of Biochemistry, Faculty of Biology, Sofia University, 8 Dragan Zankov str, 1164 Sofia, Bulgaria. Fax: 003 59 28 65 66 41. E-mail: magd@biofac.uni-sofia.bg

* Author for correspondence and reprint requests

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Embryogenic and non-embryogenic suspension cultures of orchardgrass (*Dactylis glomerata* L.) secreted into the culture medium a set of proteins, among which low molecular mass (11/12 kDa) proteins were found. However, only the 11/12 kDa proteins from the embryogenic suspension cultures reacted specifically with an antiserum raised against the carrot EP2 non-specific lipid transfer protein (nsLTP). Two-dimensional (2-D) electrophoretic analysis revealed that the extracellular nsLTP-like proteins from the embryogenic lines were acidic proteins, with pI values ranging between 4.3 and 6.4, and the 11/12 kDa proteins of the non-embryogenic lines were basic ones (pI 8–9.3). This is only the second case to report on the accumulation of extracellular acidic nsLTP-like proteins in the culture medium during somatic embryogenesis. A naïve phage display Griffin1. library was used to select single-chain phage antibodies, which specifically bind to acidic nsLTP-like proteins. Nine phage clones were selected after four rounds of biopanning of the target proteins blotted on a nitrocellulose membrane. Three soluble monoclonal single-chain phage antibodies, expressed in the non-suppressor *E. coli* strain HB2151, were purified by metal affinity chromatography and found to be highly specific for the acidic nsLTP-like proteins from the embryogenic suspension cultures. The application of the selected monoclonal antibodies for localization and elucidation of the role of the acidic nsLTP-like proteins *in vivo* is discussed.

Key words: Lipid Transfer Proteins, Somatic Embryogenesis, Recombinant Antibodies