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Review

Current potential and limitations of immunolabeling in cereal grain research

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Immunolabeling techniques have made a valuable contribution to cereal grain research during the past decade in terms of precise localization of specific compounds. While these techniques have several limitations, such as the availability and specificity of the antibodies, immunolabeling has proven especially useful in cereal studies seeking a better understanding of grain development and characterization. According to the literature reviewed in this paper, immunolabeling techniques will continue to be a useful tool in the characterization and localization of cereal grain components.

Introduction

Immunolabeling has emerged as a powerful investigative tool to localize specific cell components *in situ* within the complexity of cereal tissues and to integrate tissue-based analysis with proteomic information. Immunolabeling is a method for qualitative or quantitative determination of the presence of a target in a sample, where antibodies are utilized for their specific binding capacity. The antibodies form a complex with the target (antigen), with a detectable label being present on the antibody or on a secondary antibody. The label is a microscopically dense marker that provides a measurable signal by which the binding reaction is monitored, providing a qualitative and/or quantitative measure of the degree of binding. The relative quantity and location of signal generated by the labeled antibodies can serve to indicate the location and/or concentration of the target. The principal differences in immunolabeling methods and materials reside in the type of antibodies generated against the epitopes (monoclonal, polyclonal), the way that the label is attached to the antibody-antigen complex (direct, indirect), the type of label used (e.g., particles such as colloidal gold, fluorescent or phosphorescent compounds, and enzymes such as peroxidase or alkaline phosphatase), and the means by which the antibody-antigen complex is detected (e.g., electron microscopy, light microscopy or fluorescence microscopy).

Both direct and indirect antibody labeling are used for immunolabeling. Direct labeling utilizes only a primary antibody, which is specific for the target and is already bound to the label (Fig. 1a). This simplifies the staining procedure and provides minimal nonspecific staining and less background. Additionally, the direct labeling technique allows the use of two or more primary antibodies of the same species, avoiding the problems with secondary antibody staining. However, each different primary antibody must be tagged, which requires an abundant supply of purified antibody, and the resulting signal is weak since only one labeled primary antibody binds to each antigen. These are the main reasons why, despite the advantages of direct immunolabeling, the indirect approach is more commonly used instead. Indirect immunolabeling involves a multi-step process in which a secondary antibody bound to the label and raised against the γ globulin of the primary species is used, e.g. a goat anti-mouse antibody (Fig. 1b). Several labeled secondary antibodies can bind to each primary antibody and therefore the signal is amplified.

To date, no examples of direct labeling can be found in cereal studies. However, some alternative techniques, such as labeled enzymatic probes, have been developed for *in situ* cell wall analysis and are based on the same philosophy as direct immunolabeling (Dornez, Holopainen, *et al.*, 2011; Wilson *et al.*, 2006).

In most cereal studies, immunogold and immunofluorescence techniques are mainly applied. Fig. 2 shows examples of the application of different immunolabeling techniques to localize gluten proteins in wheat endosperm. In the case of immunogold labeling, the cell components are detected using secondary antibodies tagged with electron-dense colloidal gold particles that can be observed by transmission electron microscopy (TEM). The size of

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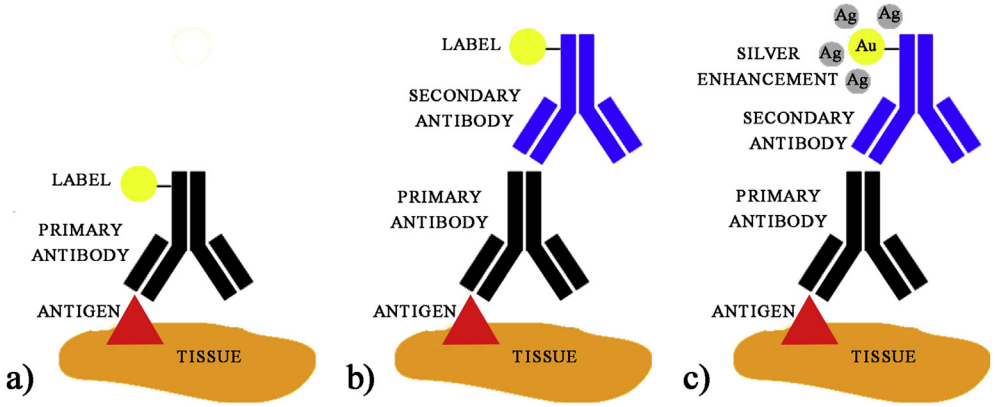

Fig. 1. Schematic representation of immunolabeling mechanisms. a) Direct labeling. b) Indirect labeling; c) Silver-enhanced indirect immunogold labeling.

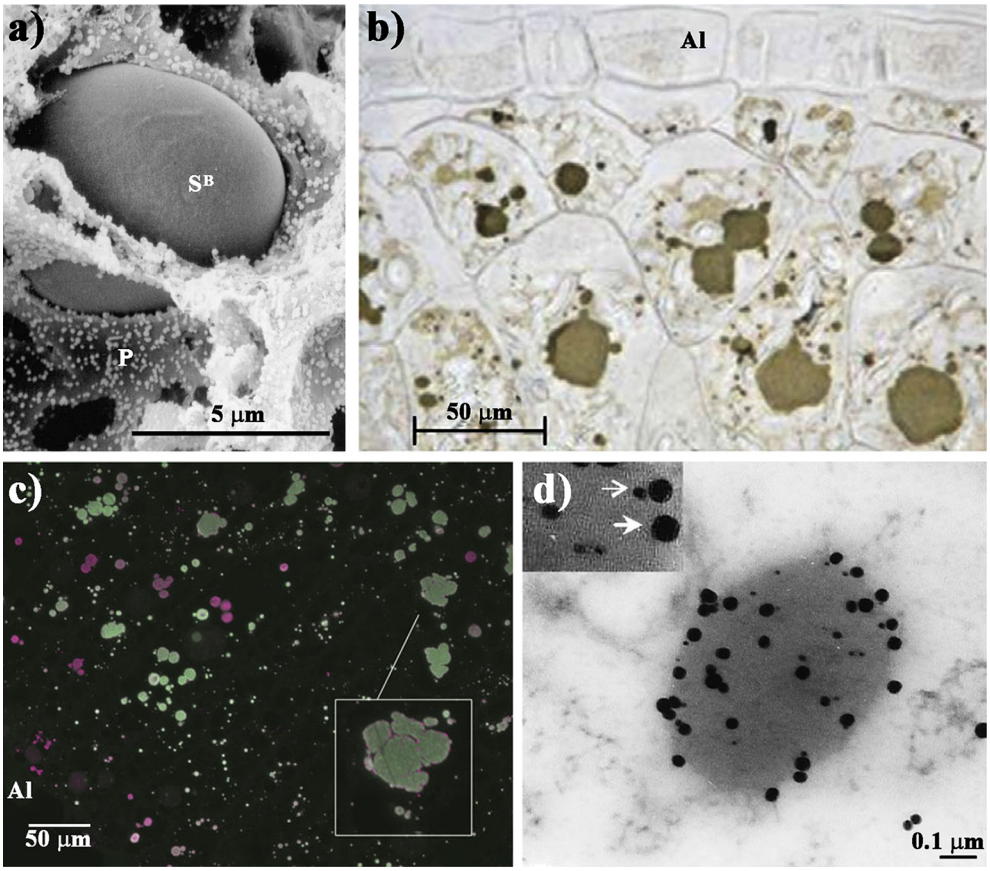

Fig. 2. Immunolocalization of gluten proteins in wheat grain endosperm using different immunolabeling techniques. a) Silver-enhanced immunogold labeling using an anti-gliadin monoclonal antibody observed by scanning electron microscopy in mature starchy endosperm cells. S^B: small starch granule; P: protein matrix (Mills *et al.*, 2005) (Reprinted from Journal of Food Science, 41, Mills, E.N.C. *et al.*, Chemical imaging: the distribution of ions and molecules in developing and mature wheat grain, pp. 193–201. Copyright 2005, with permission from Elsevier.); b) Silver-enhanced immunogold labeling using anti-α-gliadin-specific antibodies observed by light microscopy, 18 days after anthesis (daa) (Van Herpen *et al.*, 2008) (Reprinted from Van Herpen, T.W.J.M. *et al.*, Detailed analysis of the expression of an alpha-gliadin promoter and the deposition of alpha-gliadin protein during wheat grain development, Annals of Botany, 2008, 102, pp. 331–342, by permission of Oxford University Press.); c) Immunofluorescence double labeling of α-gliadin (magenta) and low-molecular weight (LMW) glutenin, 20 daa. (Tosi *et al.*, 2009) (Reprinted from Tosi, P. *et al.*, Trafficking of storage proteins in developing grain of wheat, Journal of Experimental Botany, 2009, 60, 3, pp. 979–991, by permission of Oxford University Press.); d) Double immunogold labeling observed by transmission electron microscopy. Small gold particles (empty arrows) were labeled with a monoclonal anti-α/β-gliadin antibody. Large gold (full arrows) particles were labeled with a polyclonal anti-LMW glutenin subunit antibody, 15 daa (Loussert *et al.*, 2008) (Reprinted from Journal of Food Science, 47, Loussert, C. *et al.*, Protein bodies ontogeny and localization of prolamin components in the developing endosperm of wheat caryopses, pp. 445–456. Copyright 2008, with permission from Elsevier.).