**Detection of seed pathology using tyramide amplification**

Fixed floating 40-micron sections were mounted onto gelatin-coated slides and dried at room temperature overnight. Slides were rehydrated in TBST (20mM Tris-HCl pH 7.4, 150 mM NaCl, 0.05% triton X 100) and digested with proteinase K (PK, 20 µg/mL) diluted in TBST for 20 min at 37°C. Slides were then fixed in 4% paraformaldehyde for 20 min, rinsed 3 times in TBST, and incubated with 3% hydrogen peroxide for 30 min to quench endogenous peroxidases. Slides were placed in blocking buffer (TBST, 3% bovine serum albumin, 2% goat serum) for 1h and then incubated overnight at 4°C in blocking buffer containing anti-PSER129 antibody EP1536Y (Abcam) diluted 1:50,000. The next day slides were washed 3 times in TBST and incubated with biotinylated anti-rabbit antibody (Vector Labs) diluted 1:400 in blocking buffer for 1h. Slides were washed 3 times in TBST and incubated with avidin-biotin complex (ABC) reagent (Vector labs) diluted in blocking buffer for 1h. Slides were washed twice with borate buffer (0.1M Sodium tetraborate pH 8.5) and incubate in borate buffer containing 0.003% hydrogen peroxide and 5µM biotinyl tyramide (SigmaAlrich) for 30 min. Slides were washed 3 times in TBST and incubated with ABC reagent for 1h. (Heating slides for 30 min at 80C in 20mM citrate buffer pH 6.0 before ABC reagent can increase detection sensitivity. Slides were then washed in TBST and developed using nickel-enhanced 3,3'-Diaminobenzidine DAB as previously described1. Slides were counterstained with methylgreen (Sigma), dehydrated with graded alcohols, cleared with xylenes, and cover slipped with cytoseal 60 (Fisher Scientific). Brightfield microscopy was performed using Nikon A1 laser scanning microscope. Density analysis including binary masks and region of interest (ROI) analyses were performed using Elements software (Nikon).

1 Trojanowski, J. Q., Obrocka, M. A. & Lee, V. M. A comparison of eight different chromogen protocols for the demonstration of immunoreactive neurofilaments or glial filaments in rat cerebellum using the peroxidase-antiperoxidase method and monoclonal antibodies. *J Histochem Cytochem* **31**, 1217-1223, doi:10.1177/31.10.6350434 (1983).