Detection of PjΔ6D and NcΔ15D Proteins in MON 87769 Harvested Seed By Western Blot

Introduction

The levels of PjΔ6D and NcΔ15D proteins from harvested seeds of MON 87769 grown in 2006 U.S. field trials were determined by quantifying the protein bands on western blots. This document describes how western blots were produced and presents representative western blots for both PjΔ6D and NcΔ15D proteins.

Experiment Description

Extracts from harvested seeds of MON 87769 and a conventional control with a similar genetic background (A3525) were analyzed by western blot for the presence of the PjΔ6D and NcΔ15D proteins. Both MON 87769 and A3525 samples were from seeds grown in 2006 U.S. field trials. Tissue samples were collected from plants grown from starting seed lot GLP-0604-17267-S.

Harvested seed samples were extracted in an SDA extraction buffer (100mM Tris-HCl pH 8.0, 500 mM NaCl, 10% glycerol, 2% Triton X-100, 1× protease inhibitors (Roche Diagnostics, Indianapolis, IN)) at a 1:20 tissue to buffer ratio and separated by SDS-PAGE. The extraction was done using a Harbil mixer for two, 3.5 minute cycles and insoluble material was removed from the extracts by centrifugation. The test samples were diluted in 2× Laemmli buffer while the protein reference standards were spiked into conventional seed extracts and then diluted in 2× Laemmli buffer. The reference standard was loaded at 20, 10, 5, 2.5 and 1 ng per lane for the PjΔ6D protein and 75, 50, 25, 10, 5, 2, and 1 ng per lane for the NcΔ15D protein. Additionally, Precision Plus Protein Standards (Bio-Rad, Hercules, CA) were loaded on the gel to demonstrate the transfer of proteins to the membrane and to determine the approximate molecular weight of both proteins. Except for the reference standards, all samples were loaded in triplicate.

Proteins separated by SDS-PAGE were electrophoretically transferred to Criterion Nitrocellulose membrane (Bio-Rad) or Invitrolon PVDF membrane (Invitrogen, Carlsbad, CA) using 1× Tris-Glycine transfer buffer (Bio-Rad) or 1× Novex Tris-Glycine transfer buffer (Invitrogen) containing 20% methanol. After transfer, non-specific sites on the membrane were blocked using 5% (w/v) non-fat dried milk (NFDM, Bio-Rad) in Phosphate -Buffered Saline with 0.05% (v/v) Tween-20 (1× PBST) or Tris-Buffered Saline with 0.1% (v/v) Tween-20 (1× TBST). The membranes were then probed with PjΔ6D- or NcΔ15D-specific polyclonal antibodies at a 1:2500 dilution. Bound antibodies were detected with a 1:5000 dilution of anti-goat IgG antibody conjugated to horseradish peroxidase (Pierce, Rockford, IL) in 1× PBST with 1% (w/v) NFDM or 1× TBST with 5% (w/v) NFDM. Unbound anti-goat IgG-HRP antibodies were removed by rinsing the membrane briefly and then washing four times for 10 minutes each in 1× PBST or 1× TBST. The SuperSignal West Pico Chemiluminescent Substrate (Pierce) was added to the membrane according to the manufacturers' instructions. The membrane was exposed to Hyperfilm ECL (Amersham) to record an image of the immunoreactive bands.

Figures 1 and 2 show representative western blots of extracts from harvested seed of MON 87769 and the conventional control, where the PjΔ6D and NcΔ15D proteins were detected.

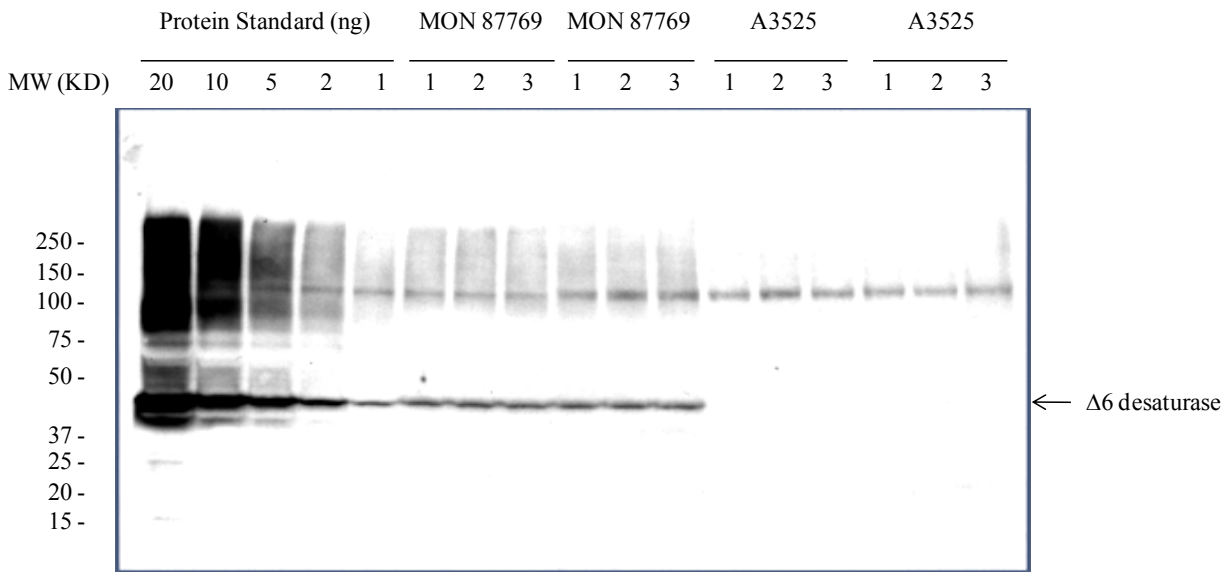

Figure 1. Representative western blot of the PjΔ6D protein in extracts from harvested seed of MON 87769 and the conventional control A3525.

Harvested seed samples were extracted in an SDA extraction buffer at a 1:20 tissue to buffer ratio. Seed extract supernatants were diluted in 2× Laemmli buffer prior to SDS-PAGE. Conventional seed extract was added to the reference standards for each protein and diluted in 2× Laemmli buffer. All samples were loaded in triplicate except for the reference standards.

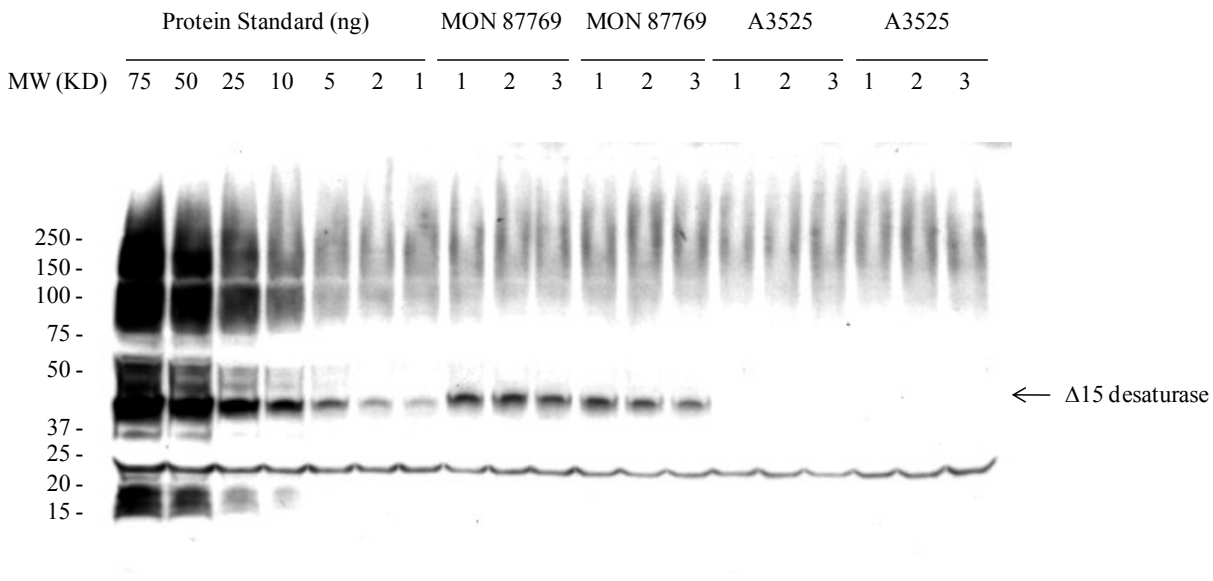

Figure 2. Representative western blot of the NcΔ15D protein in extracts from harvested seed of MON 87769 and the conventional control A3525.

Harvested seed samples were extracted in an SDA extraction buffer at a 1:20 tissue to buffer ratio. Seed extract supernatants were diluted in 2× Laemmli buffer prior to SDS-PAGE. Conventional seed extract was added to the reference standards for each protein and diluted in 2× Laemmli buffer. All samples were loaded in triplicate except for the reference standards.