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**STUDY TITLE**

Efficacy of Reduced Polyphenol Oxidase Activity in Z6 Tubers compared to Snowden at  
Harvest and After Storage

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**STUDY NUMBER**

[CCI]

### **Certification Page**

I, the undersigned, declare that, to the best of my knowledge, this report provides an accurate evaluation of data in this study.

Signed \_\_\_\_\_

[personal information redacted]  
Regulatory Affairs Manager

14 March 2019 \_\_\_\_\_

Date

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## Summary

**Objective:** In this study, polyphenol oxidase (PPO) activity of Z6 tubers was compared to Snowden (SN) tubers to demonstrate PPO trait efficacy during storage.

**Methods:** Polyphenol oxidase activity was measured by monitoring the rate of appearance of the red dopachrome pigment. Freeze-dried, ground tuber samples from Z6 plants were compared with Snowden controls. Tubers freshly harvested and stored for six months in 45 °F storage were compared.

**Results:** Ground tubers from fresh and stored Z6 plants were compared to Snowden using a PPO activity assay. Addition of PPO substrate to ground Snowden tubers led to a significant increase in PPO activity, whereas little PPO activity was measured in any of the Z6 tuber samples. These data demonstrate that Z6 trait efficacy associated with a reduction of PPO activity in Z6 tubers was stably maintained during six months of storage.

**Conclusion:** A reduction in PPO activity after six months was measured in tubers from Z6, indicating trait efficacy remained stable after storage.

## Introduction

Z6 was generated by transforming Snowden with pSIM1278 and pSIM1678 using *Agrobacterium* transformation. One of the traits introduced was reduced black spot, achieved by a reduction in polyphenol oxidase (PPO) activity through RNA interference (RNAi).

Black spot is a post-harvest physiological phenomenon resulting from the handling of potato tubers during harvest, transport, and processing, and refers to the black or grayish color that may form in the interior of damaged potatoes. The enzymatic darkening and discoloration results when PPO is released from plastids of damaged potatoes and ultimately converts cytosolic diphenolic compounds (e.g. chlorogenic acid) into melanin, forming the dark pigment associated with black spot. Potatoes that show black spot are typically trimmed, or the entire potato is rejected before processing. This results in quality control challenges, economic loss, or both.

Decreased PPO activity in Z6 tubers reduces the occurrence of black spot, which reduces potato waste.

In this study, PPO activity was measured by monitoring the rate of appearance of the red dopachrome pigment. The PPO activity was measured in Z6 and Snowden fresh tubers, and in those stored at 45 °F for six months.

## Study Objectives

The objective of this study was to assess the reduced polyphenol oxidase activity in stored Z6 tubers.

## Study Dates

09/2018 – 03/2019

## Key Personnel

[personal information redacted]

## Materials and Methods

### Field Trials and Storage

During the growing season of 2018, Z6 and Snowden were grown in Arena Valley, Idaho. The agronomic practices and pest control measures were location-specific and typical for all aspects of potato cultivation including soil preparation, fertilizer application, irrigation, and pesticide-based control methods. Z6 and Snowden tubers were stored in a storage facility at 45 °F for six months in Caldwell, Idaho.

### PPO Activity Assay

Four biological replicates were processed from Z6 and Snowden plants. Six tubers were collected from each Z6 replicate and from each Snowden replicate. [CCI]

Z6 and Snowden fresh tubers were processed as described above and aliquots of these Z6 and Snowden samples were analyzed immediately [CCI]. These freeze-dried, ground Z6 and Snowden samples were then stored at room temperature for the same amount of time as the Z6 and Snowden tubers were stored at 45 °F. Aliquots of these freeze-dried, ground, fresh Z6 and Snowden samples (Fresh Z6 and Fresh SN) were analyzed alongside the processed Z6 and Snowden stored tubers (6 months Z6 and 6 months SN).

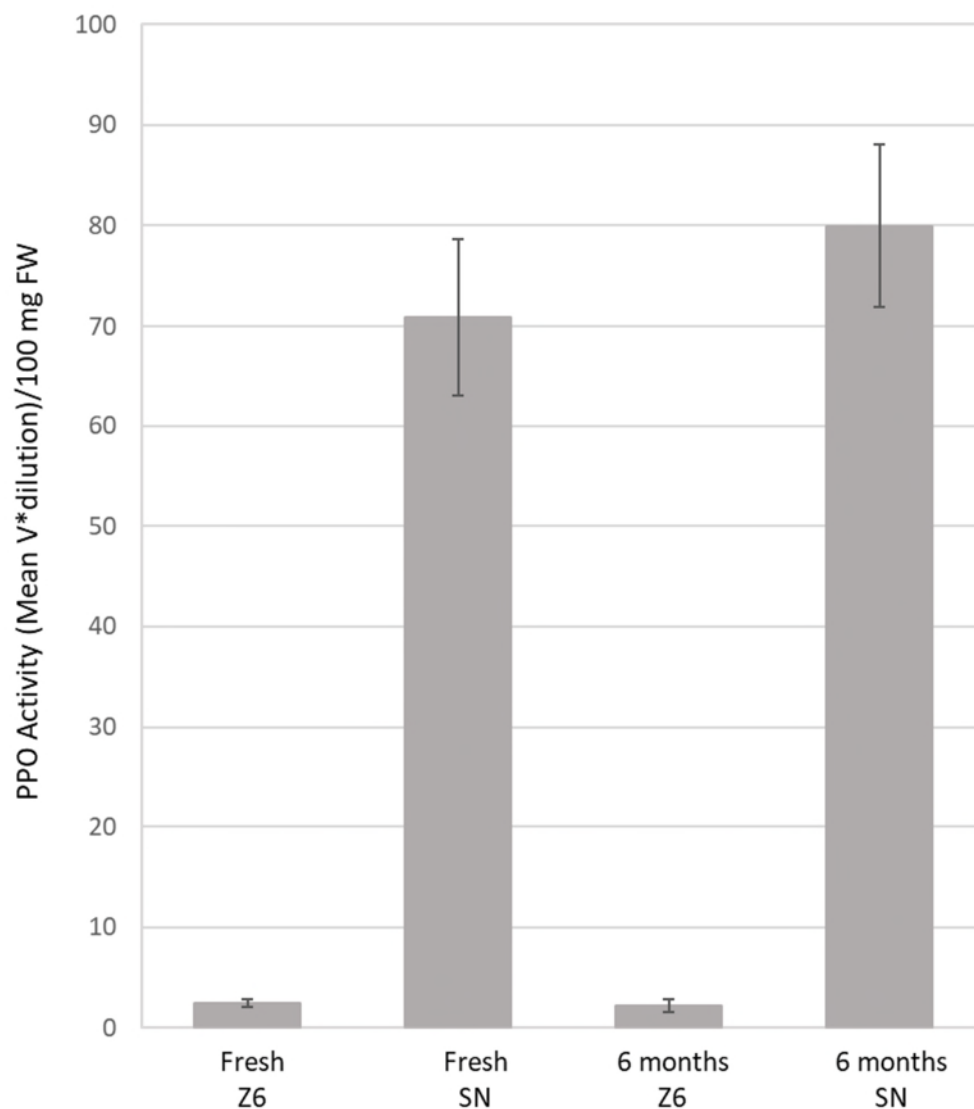
Thirty mg of freeze-dried tuber from each sample was weighed into a microcentrifuge tube for analysis. [CCI]

## **Results**

Oxidation of diphenolic compounds catalyzed by PPO results in formation of o-quinones, leading ultimately to the formation of melanin, which can lead to dark spots in the tuber (Behbahani et al., 1993). The rate of polyphenol oxidase activity can be measured by monitoring the disappearance of the exogenous substrate (L-DOPA) or by monitoring the appearance of the product, dopachrome (see below for reaction). In this study, PPO activity was measured by monitoring the rate of appearance of the red dopachrome pigment. This assay was used to verify trait efficacy in Z6.



The PPO activity was compared in fresh and stored tubers from Z6 plants to Snowden (Figure 1). These data indicated that PPO activity was very low in Z6 plants relative to Snowden and that the trait remained stable over the six month storage time period. Less PPO activity results in less dark spot formation in Z6 tubers compared to conventional Snowden tubers.



**Figure 1. Reduced PPO Activity in Z6 Tubers**

PPO activity assay performed on fresh and stored Z6 and Snowden (SN) tubers. Low PPO activity was observed in Z6 compared to the SN conventional tubers. PPO activity was calculated by taking the average of four biological replicates.

## Conclusion

Compared to Snowden, PPO activity was reduced in Z6 tubers and remained reduced after six months of storage, which is consistent with effective down regulation of PPO. Decreased PPO activity in Z6 tubers reduces the occurrence of black spot.

## References

Behbahani, I., Miller, S., and Okeeffe, D. (1993). A Comparison of Mushroom Tyrosinase Dopachrome and Dopachrome Assays Using Diode-Array Spectrophotometry: Dopachrome Formation vs Ascorbate-Linked Dopachrome. *Microchemical Journal* 47, 251–260.