The relationship between post-harvest storage conditions and membrane leakage in the context of Blackheart in potato tubers

Yang Yue
University of Florida

Faculty mentor: Bala Rathinasabapathi, Department of Horticultural Sciences

Abstract

The relationship between potato (Solanum tuberosum L.) tuber membrane permeability and storage conditions were examined. Tubers from four potato cultivars were stored for one year at 2 °C and 4 °C, and tubers transferred from 4 °C to 2 °C for 48 hours. Electrolyte conductivity measurements were taken from two discs of four potato cultivars with six biological replicates as well as different tissues types including the pith, cortex, and parenchyma. The results showed that the longer the storage time and lower the temperature, the higher the electrolyte conductivity of the tissues. Moreover, different tissue types pith, cortex, and parenchyma had different electrolyte conductivity susceptibility pattern under different storage conditions. Overall, the parenchyma has the highest electrolyte leakage, and following are pith, cortex has the lowest among three different tissue types. However, cultivars that are resistant to Blackheart surprisingly had higher electrolyte conductivity compared to the Blackheart-susceptible cultivars. Overall, the work demonstrates that storage conditions have a significant effect on membrane leakage but that this doesn’t appear to relate directly to susceptibility to Blackheart.

Keywords: potato tubers, blackheart, membrane leakage, storage temperature

Introduction

Potatoes are of great nutritional and economic value, and they have been planted and distributed worldwide. However, potato waste due to postharvest issues is a significant cause of crop loss in both developing and developed countries (Hodges et al., 2011). In the UK potato industry, one of the most important postharvest issues is the physiological disorder known as Blackheart that leads to dark pigmentation in the center of the tuber (Leon, 2015). This damage causes considerable economic and nutritional loss from producers and consumers perspective. Blackheart pigmentation is linked to polyphenol oxidase (PPO) enzyme activity that is in turn associated with the loss of subcellular compartmentalization: loss of integrity of intracellular membranes allows mixing of PPO (localized mainly within plastids) and phenolic amino acid substrates that are mainly accumulated in the vacuole (Vámos-Vigyázó & Haard, 2009). The
phenolic amino acids are oxidized to $o$-quinones which are non-enzymatically oxidized to dark brown-black polyphenols, such as melanins.

Blackheart is correlated with temperature stress and the length of the storage period (Davis, 1926). One possible mechanism underlying Blackheart is that long-term cold-storage leads to loss of membrane integrity, but this has not yet been systematically tested. Accordingly, this work addresses the hypothesis that cold temperature has a positive correlation with cell membrane leakage and that this in turn correlates with Blackheart susceptibility. Specifically, the lower the temperature, the higher the cell membrane leakage. Additionally, I propose that the length of the storage period also may have a positive correlation with the cell membrane leakage. To test these hypotheses, measurements of cell membrane integrity using electrolyte leakage were made in tubers stored at different temperatures and for different lengths of time. To relate these changes to Blackheart susceptibility, electrolyte leakage was monitored in three tuber tissue types: pith, parenchyma and cortex, with the pitch and parenchyma being the sites of Blackheart and while Blackheart is never found in the cortex. Additionally, I will take advantage of previous research that has identified potato cultivars that have differing susceptibility to Blackheart (Lottie Chapman, University of Oxford, personal communication).

**Materials and Methods**

**Plant Material andnd Storage Conditions**

Four different potato cultivars were used: two cultivars that are resistant to Blackheart, Maris Piper (MP) and Chopin (CH), and two susceptible cultivars, King Edward (KE) and Marfona (Ma). They were stored in the dark at 4 °C and 2°C, respectively, for one year. For some experiments, the potato tubers were transferred from 4°C to 2°C for 48 hours prior to measurements.

**Electrolyte Conductivity Measurement**

A conductivity meter was used to measure the electrolyte leakage from tuber tissues. Discs of 9 mm diameter were cut with a cork borer from the pith, cortex and parenchyma part. The discs were uniformly cut by using 9mm punch. The background conductivity of 5 ml deionized Milli-Q water was measured, before two discs of each tissue were incubated in the water for 5 min with shaking. The conductivity of the solution was then measured again. Finally, the tube containing the tissue and solution was placed in the minus 80 °C freezer to freeze the tissues and
completely break the cell membrane. The tube was thawed and a final conductivity measurement was taken. The percentage of electrolyte was calculated as follows: A indicates the background conductivity of distilled deionized water, B indicates conductivity after 5-minute shaking measurement of tissue leakage, C indicates after minus 80 °C storage measurements of tissue leakage. The formula is as follow: \((B - A)/(C - A) \times 100\%\) = The percentage of tissue electrolyte leakage.

**Statistical Analyses**

Quantitative data were plotted and evaluated by calculating means and standard errors. The analysis of variance was done by using the SAS statistical package (version 9.4; SAS Institute, Cary, NC) and significant differences between means at \(P = 0.05\) calculated using Duncan’s multiple range test or Fisher’s LSD test at 5% significance level.

**Results**

**Membrane leak in tuber tissues Under 4 °C storage for one year**

In terms of electrolyte conductivity of potato tuber tissue type including pith, cortex, and parenchyma, the resistant cultivar MP had a significantly higher electrolyte leak rate than the susceptible cultivars KE and MA, showing 35% and 37% increased leak, respectively, compared to the two susceptible cultivars. The resistant cultivar CH and MP had a significantly higher cortex tissue electrolyte leak rate than the susceptible cultivar KE, showing 38% and 63% increased leak, respectively, compared to the susceptible cultivar KE. Both resistant cultivars CH and MP had a significantly higher parenchyma tissue electrolyte leak rate than both susceptible cultivar KE and MA, showing 22% increased leak, compared to the average leak rate of the two susceptible.

**Membrane leak in tuber tissues Under 2 °C storage for one year**

A different pattern of electrolyte leakage was seen in tubers stored at 2 °C. Electrolyte leakage was slightly higher in all cultivars compared to storage at 4 °C. Additionally, the pattern between the different cultivars was altered. The electrolyte conductivity of potato tuber tissue type about pith and parenchyma shared a similar pattern compared with storage under 4 °C for one year. The resistant cultivar MP had significantly higher electrolyte rate, showing a 16% increased leak, compared to the susceptible cultivar MA. On the contrary, the resistant cultivar CH had significantly lower electrolyte rate, showing a 19% decreased leak, compared to the
susceptible cultivar KE. However, there was no significant difference between both resistant and susceptible cultivars in terms of cortex membrane electrolyte leak.

**Membrane leak in tuber tissues transferred from 4 °C to 2 °C storage for 48-hour**

Compared to another two tuber tissues storage conditions under 4 °C for one year and 2 °C for one year, membrane leak in tuber under 2 °C was transferred from 4 °C for 48 hours showed slightly lower in all cultivars compared to the previous conditions solely storage at 4 °C and 2 °C. Specifically, the resistant cultivar MP had a significantly higher electrolyte, showing 28% and 3% increased leak, compared to the susceptible cultivars KE and MA. The resistant cultivar MP had higher electrolyte rate, showing 49% increased leak, compared to the susceptible cultivar MA. It also shared the same pattern of parenchyma tissue membrane leak as potato tuber stored at 4 °C for one year. The resistant cultivar CH and MP had significantly higher electrolyte rate, showing 29% increased leak compared to the susceptible cultivars KE and MA.
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Figure 1. The electrolyte conductivity of different potato tuber tissue types under different storage period. The measurements took by a conductivity meter. Mean values (n=6) not significantly different at p=0.05 are followed by the same letter code using Duncan’s multiple range test. Statistical analyses were done for comparing means for each tissue type from different cultivar. Those mean values resistant cultivars (Maris Piper (MP) and Chorpin (CH)) are significantly different from susceptible cultivars (King Edward (KE) and Marfona (MA)) are shown in different letters. Membrane leak in tuber tissues after 4°C storage for one-year A) Membrane leak in tuber tissues after 2°C storage for one-year B) Membrane leak in tuber tissues transferred from 4°C to 2°C storage for 48 hours.

To understand the susceptibility of different potato tubers, including pith, cortex, and parenchyma in response to temperature changes and storage period. We calculated the electrolyte leak proportion between different tissue types such as Pi/Co (Pith/ Cortex), Co/Pa (Cortex/Parenchyma), and Pi/Pa (Pith/Parenchyma).

Different tuber types under 4°C for one-year storage

The resistant cultivars CH and MP had significantly lower proportion of pith and cortex, showing 25% and 23% decreased proportion, respectively, compared to susceptible cultivar KE. The resistant cultivar MP had significantly higher proportion of pith and parenchyma, showing 16% increment compared to the average proportion of the two susceptible cultivar KE, and the proportion of cortex and parenchyma followed the same pattern that resistant cultivar MP had significantly higher proportion and showed 40% and 19%, respectively, increased compared to the susceptible cultivars KE and MA.

Both resistant cultivars have higher electrolyte conductivity in pith, cortex, and parenchyma compared to other two susceptible cultivars. Both Pith and Parenchyma of susceptible cultivars have higher electrolyte conductivity than cortex compared to resistant cultivars.

Both susceptible cultivars have higher electrolyte conductivity in Pi/Co compared to other two resistant cultivars. Both resistant cultivars have higher Co/Pa compared to Pi/Co and Pi/Pa than other two susceptible cultivars.
Different tuber types under 2 °C for one-year storage

Compared to the tuber tissue stored at 4 °C, the resistant cultivars CH and MP was seen no significantly different from susceptible cultivars KE and MP in both proportion pith and cortex, and proportion cortex and parenchyma. However, the proportion of pith and parenchyma shared the similar pattern with the potato tuber stored at 4 °C which both resistant cultivars CH and MP had significantly higher proportion and increased by 20% compared to the susceptible cultivars KE and MA.

In terms of electrolyte conductivity of potato tuber tissue type, there is no significant difference between resistant and susceptible cultivars in terms of Pith and Cortex conductivity. However, the susceptible cultivars KE and MA is significantly different from the resistant cultivar CH and MP.

In terms of electrolyte conductivity proportion of potato tuber tissue, there is no significant difference between the resistant cultivar and susceptible cultivar in terms of the Pi/Co proportion. There is a significant difference between the resistant cultivar and susceptible cultivar in terms of Pi/Pa and Co/Pa proportion.

Different tube types under transferred from 4 °C to 2 °C for 48-hour storage

Compared to the tuber stored at 2 °C, the resistant cultivars CH and MP and susceptible cultivars KE and MA had no significant difference in both proportion pith and cortex and proportion of cortex and parenchyma. As far as the proportion of pith and parenchyma, it has the opposite pattern followed the resistant cultivar MP had significantly lower proportion and decreased by 13% compared to the susceptible cultivar MA.
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Figure 2. Comparisons of the electrolyte conductivity proportion between different potato cultivars and different tuber tissue under different storage temperature. (A) 4 °C one-year storage. (B) 2 °C for one-year storage. (C) transferred from 4 °C to 2 °C for one-year storage. Mean values (n=6) not significantly different at p=0.05 are followed by the same letter code using Duncan’s multiple range test. Statistical analyses were done for comparing means for each tissue type from different cultivar.

Figure 3. The morphological changes on the cut section of potato tuber showed Blackheart Picture retrieved from https://potatoes.ahdb.org.uk/media-gallery/detail/13213/3355

Discussion

As figure 3 shown, Potatoes blackheart is characterized by the color changes and crack-like cavity formation are observed in the center, initially without showing symptoms on external. It is a non-pathogenic disorder which differentiated from bacterial or fungus causation, driving internal brown to black discoloration (Davis, 1928). This process has accompanied a series of
physiology and biochemical changes in the course of blackheart formation. During the postharvest handling process, the fact is potatoes are still living tissues where a lot of metabolic activities are undergoing. For example, respiration is the major energy source that utilizes the carbohydrates stored in the sink to keep the tissues and cells alive although detached from the plants, respiration rates are heavily dependent on the storage temperature. Generally, potatoes best keep at temperatures arranging from 38 °F to 45 °F. For fresh market potatoes, the optimum storage temperature is 40 °F (4 °C or so) (Voss et al. unknown).

In terms of objective of this experiment, (1) We try to demonstrate blackheart induction under long term acclimation to 4 °C versus 2 °C in different potato varieties;

We used conductivity meter and measured the electrolyte leakage of 4 different varieties under two storage temperatures. By measuring the electrolyte leakage, we could get an idea of different degrees of cell membrane integrity in response to cold impairments both resistant and susceptibility cultivars; this potentially leads us to further explore the driving factors for blackheart formation. As figure 1 (A) shown, we found the cell membrane leakage are heavily influenced by the storage temperature but not cultivars dependent. However, under the 2 °C, as figure 1(B) pointed out, all tissue types have higher electrolyte leakage compared to the storage under 4 °C for a year. This result proves to be potatoes have severe damage under lower storage temperature which below the optimum storage. However, the higher value of electrolyte leakage kind of contradicts with the fact that temperature reduction will decrease the rate of all metabolic reactions. However, this confirmed that initial storage temperature (1.5°C) correlated the Blackheart incidence is higher than warmer temperature (3.5°C) (Briddon, 2015). To further tested the that potatoes tubers whether needs long term acclimation to 2 °C storage, we also took the measurement of electrolyte leakage of different tubers tissues types that transferred from 4 °C to 2 °C for 48 hours. As figure 1(C) indicated, we found that both resistant and susceptible cultivars have the lowest electrolyte leakage among three different storage conditions, which means potato tubers could not immediately acclimate to the new storage condition (2 °C).

Besides, Blackheart resistant and susceptible potato tubers acted differently under different temperature storage conditions. Under 4 °C storage for one-year condition, the resistant cultivars have significantly higher electrolyte leakage compared to susceptibility cultivars. This against the tradition that resistant cultivars normally are more robust in response to the chilling stress conditions, which has less damage and lower electrolyte leakage. This is also the case for the
potato tubers that transferred from 4 °C to 2 °C for 48 hours. Expect for that both resistant cultivars have lower electrolyte leakage than susceptible cultivars in terms of parenchyma tissue types. One possible explanation for that exception is Blackheart development extends from the central tissues to the periderm internally along with medullary rays towards the buds/eyes. Parenchyma is similar to transition zone along with the discoloration progress. Under cold stress acclimation, the resistant cultivars potentially prone to have less damage to Blackheart compared to the susceptible cultivars. In the case of potato tubers transferred from 4 °C to 2 °C which followed the same pattern that Blackheart could not respond to temperature acclimation right away. This probably indicated that there is no direct correlation between potato tuber cultivars and electrolyte leakage under recommended storage temperatures (4 °C), Blackheart progression related to the electrolyte leakage is a gradual process under the cold stress acclimation conditions.

We also calculated the ratio of different tissue types which represented by the proportion of 4 cultivars under separate temperature storage conditions. As figure 2 (A) shown, for potato tuber stored at 4 °C for one year, we found there is no stringent correlation exits in the proportion of different tissue types between resistant cultivars and susceptible cultivars. However, in figure 2 (B) the resistant cultivars have higher the proportion of Pi/Co, Pi/Pa, Co/Pa than the susceptible cultivars under the 2 °C stored for one year. Conversely, as figure 2 (C) shown indicated in the susceptible cultivars have higher the proportion of Pi/Co, Pi/Pa, Co/Pa than the resistant cultivars under the 4 °C were transferred to 2 °C. This may reveal the susceptibility of different tissue types to cold storage exhibited differently between resistant versus susceptible to Blackheart. And the opposite results may indicate the different tissue types have different level of severity that responds to the cold stress in resistant cultivars. The dramatic changes probably indicated different part of tissues experienced the electrolyte leakage in an uneven manner. The Blackheart pigmentation variations appear not to be a diffusion gradient since sometimes black discoloration may appear to perimedullary area instead of the pith tissues. (Kiaitsi, 2015; Hooker, 1981).

The correlation of PPO activity and cell membrane death associated with Blackheart.

Previous research demonstrated that Blackheart may be caused by lack of oxygen or excessive CO2 concentration. This process is not only involved in physiological occurrence, but also happened alongside with biochemical reactions. Mainly an enzyme-substrate complex
activity which determined by the subcellular compartmentation that initiated the PPO activity. PPOs are widespread enzymes in plants and some other microorganisms, it is advantageous to trigger defense mechanism to predations and pathogenies (Steffens et al., 1990; Mayer, 1987; Thygesen, 1995) however, from post-harvest handling and consumers standpoint, it may negatively exert influences on economic loss (shelf life of fresh produce) as well as postharvest attributes such as color, nutritional prosperities (Taranto et al., 2017).

There are two major factors are significantly affecting the potato tuber PPO activities which are PH and temperatures. Potato tuber shows two PH optima that are 4.5 and 5.0. The substrate of PPO stored in the vacuole this acid enclosed environment and PPO are enzymes that localized in plastids specifically amyloplasts this membrane-bound organelle. During the postharvest, the mechanic injury bruising, chillily storage, or programmed cell death all leads to the breakdown of cell membrane integrity, this probably leads to the PPO physically and chemically interacting with substrates and function as catalytic to accelerate oxidation activities. Finally, brown complex polymers known as melanins are formed. Another non-enzymatic reaction also can directly oxidase the o-quinones to dark polyphenols melanins, this process largely owing to the highly reactive attributes of o-quinones in the presence of oxygen. (Taranto et al., 2017)

In our experiment, we found that under the recommend temperature storage conditions, resistant cultivars generally have more leaky membranes compared to susceptible cultivars, however it exhibits a similar pattern that resistant cultivars CH and MP also have more leaky membranes under the 2 °C for one-year as well as retransferred from 4 °C to 2 °C for 48-hours. This unusual observation showed the Blackheart resistant cultivars have either plastid or vacuole is more vulnerable to the cold stress compared to the susceptible cultivars, another possible explanation is that the concentration of PPO in the amyloplasts or the concentration of substrate in the vacuole are higher compared to the susceptible cultivars. Then this happened to have more affinity to form the enzyme-substrate complex and causes greater reaction rate of PPO activity.

Besides, we generally found the proportion of Cortex and Parenchyma has the lowest values compared to the largest proportion of Pith and Cortex, and the second largest proportion of Pith and Parenchyma. This also stressed the parenchyma tissues have the highest membrane leakage compared to the pith. However, cortex tissues have the least damage to cold impairments. Other than PPO activity that contributes to the Blackheart, a small multigene family has also been isolated from the amyloplasts of the tuber cell that responsible for differential expression could
also potentially leading to high PPO activity. (Thygesen, 1995), another possible explanation for this is the spatial expression of genes has detected differently expressed in all parts of tubers and throughout tuber development. This also probably addressed that different tissue types showed different levels of membrane leakage among the four cultivars under three kind of storage conditions.

Potato tubers are highly metabolic active during the storage, the rate of respiratory activity was very high and gradually dropped to a relatively constant level in a period of three to six weeks. (Schippers, 1977). The living potato tubers go through respiration process and take in oxygen from the environment; gases gradually diffuse from external cortex to internally pith along the gradient. Potato tuber stored at below 7 % $O_2$ causes no gradient that hardly diffuses into the tuber’s tissues (Jeff Brecht, University of Florida, Personal Communication). Oxygen is more easily accessible to the cortex compared to the pith, so the central tissues pith will easily be absent from oxygen. When oxygen becomes limiting factor that decreases external oxygen concentration, making plant tissues could not keep pace with the rate of oxygen consumption, this is where electron acceptors under the cold stress conditions (Geigenberger, 2003), Reactive Oxygen Species (ROS) intracellular production tend to have effects on cell metabolism such as oxidation of polyunsaturated fatty acids in lipids, amino acids in proteins, co-factors that deactivated specific enzymes. (Brooker, 2011) This probably demonstrated that internal tissue types pith and parenchyma had more cell membrane damage than the cortex.

**Conclusion**

Based on the discussion, we draw the conclusion that cold temperature and storage period could be the major driving factors contributing to the physiological disorder, but it may not directly associate with Blackheart, and Blackheart resistant cultivars and susceptible cultivars have no distinct correlation associated with environmental factors shifting. This work has characterized the temperature acclimation response of modern potato varieties, by identifying the underlying physiological changes in tubers during Blackheart development help us to understand the driving forces of the disorder. This could potentially provide suggestive recommendations for post-harvest industry to handle the storage environment as well as reduce disorder occurrence. Most importantly, it would contribute to reduce food waste in the industry. In the future, we could measure the respiration rates of different cultivars tubers and understand whether some changes take place in blackheart resistant cultivars versus susceptible cultivars.
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