THESIS

THE INFLUENCE OF GENOTYPE, ENVIRONMENT, AND STORAGE TIME ON THE ASCORBIC ACID CONTENT AND RETENTION IN POTATO GERMPLASM FROM THE COLORADO POTATO BREEDING AND SELECTION PROGRAM

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ABSTRACT

THE INFLUENCE OF GENOTYPE, ENVIRONMENT, AND STORAGE TIME ON THE ASCORBIC ACID CONTENT AND RETENTION IN POTATO GERMPLASM FROM THE COLORADO POTATO BREEDING AND SELECTION PROGRAM

Potato is a globally consumed vegetable crop known to contain vitamin C, with its active form, ascorbic acid (AsA), serving as a potent antioxidant involved in numerous physiological processes within the human body. The oxidized form, dehydroascorbic acid (DHA) was not measured in this study. Thus, the focus of this thesis was to investigate ascorbic acid in potato germplasm in the Colorado Potato Breeding and Selection Program.

However, even if a potato genotype contains a sufficiently large amount of AsA, immediately after harvesting, its content may significantly decrease during storage. Therefore, it is so important to focus not only on the initial AsA content but also on its retention in storage.

An investigation was conducted to enhance our understanding of the potential to increase AsA content in potato tubers through traditional breeding. This study examined the variations in AsA levels due to genetic factors (assaying multiple genotypes), environmental conditions (different growing locations), and AsA retention (sampling during storage). This study was divided into 2 parts.

In Part 1 (Year 1, 2021), AsA initial level and its retention during storage was investigated in 34 genotypes grown in the San Luis Valley, CO, USA. The initial AsA content ranged from 8.5 to 37.7 mg/100 g FW. All genotypes experienced some level of AsA loss during storage, with the mean loss across all 34 genotypes being 34.8%. Notably, there was considerable variation in both initial AsA levels and retention among the genotypes, with some even exhibiting a temporary increase in AsA content during storage.

In Part 2 (Year 2, 2022), six cultivars (selected from 34 from last year) were grown in three different locations to investigate the effect of environmental conditions on the initial content of AsA and retention. Among the genotypes examined, three showed evidence of variation between AsA retention and growing location (time:environment, TxE interaction), indicated by varying slopes. Four genotypes demonstrated variation in initial AsA content over three different locations, representing a genotype:environment, (GxE) interaction.

In conclusion, this investigation emphasizes the potential for improving potato tuber AsA content through traditional breeding, while also underscoring the significance of considering both the initial content and retention during storage to maximize nutritional benefits. This research highlights the complex interactions between genetics (genotype), environment (growing location), and storage time that influence AsA retention in this widely consumed vegetable.

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER 1 INTRODUCTION

Potato (Solanum tuberosum, L.) is the fourth most produced major staple crops in the world along following rice, wheat, and corn, with the consumption of 49.4 pounds of potatoes per capita in 2019 (Western Regional Trial Reports, USDA). It is the most consumed vegetable worldwide, known for its high caloric and nutritional density per unit of production, as well as its excellent post-harvest storage characteristics and food processing properties.

Potatoes, as an easily accessible source of calories, are rich in various useful nutrients and bioactive compounds, including vitamin C. This vitamin comes in two forms: active and passive. Both play distinct roles in the human body. The active form, AsA, serves as a potent antioxidant and a cofactor, participating in numerous functions such as protection against oxidative stress, stimulation of certain enzymes, collagen biosynthesis (Pinnell, 1985), hormonal activation, histamine detoxification, and the phagocytic functions of leukocytes (Carr & Rowe, 2020). It has also been associated with reduced blood pressure, enhanced immunity, drug metabolism, and tissue regeneration (Walingo, 2005). The current US recommended daily allowance (RDA) for AsA ranges between 100–120 mg per day for adults (Naidu, 2003). Consequently, including AsA-rich potatoes in the diet may reduce the need for AsA dietary supplements.

There are many factors working either together or independently which affect AsA level in potato tubers. Studies have shown that one of the major factors affect the phytochemical and metabolic composition of potatoes are genotype and environmental conditions (Burgos et al., 2009; Goyer et al., 2019; Samaniego et al., 2020) whereas genotype accounted for the greatest variation in the phytochemical composition (Samaniego et al., 2020). This supports the idea that AsA content can be potentially increased in commercially available cultivars through traditional breeding or other forms of genetic improvement with the use of commonly available potato germplasm as it was discussed in multiple studies (Lovat et al., 2016; Love & Pavek, 2008; Stushnoff et al., 2008).

Potatoes, as a vegetable, can be stored for several months before reaching consumers. During this time, they remain physiologically active, with ongoing metabolic processes. Numerous studies have indicated that the AsA content in potatoes undergoes degradation during long-term storage demonstrating some fluctuations in degradation patterns under varying storage temperatures (Goyer et al., 2019; Oktay, 2013; Yamdeu Galani et al., 2017). Collectively, even if a potato genotype contains a sufficiently large amount of AsA, immediately after harvesting, its content can significantly decrease before it reaches the consumer's table (Alamar et al., 2017; Burgos et al., 2009; Demidenko et al., 2021; LEŠKOVÁ, 2006; Oktay, 2013; Tudela et al., 2002). Therefore, it's important to understand variations in both initial AsA content and AsA retention.

CHAPTER 2. REVIEW OF LITERATURE

2.1. Nutrient Deficiency in Humans

Plant foods provide important phytochemicals classified as micronutrients, which are dietary components that are essential for human health. Vitamins are a sub-class of micronutrients that are small molecules with various roles in human physiology, molecular biology, and development. Insufficient intake of these vital nutrients is referred to as malnutrition and can lead to the development of severe disorders that affect an individual's physical well-being and overall health.

To address micronutrient deficiencies, two primary strategies have been implemented: supplementation and biofortification. According to Food and Drug Administration (FDA) supplementation refers to the consumption of orally ingested products containing "dietary ingredients" with the aim of complementing or augmenting the diet which is usually associated with additional costs for the consumer. Whereas biofortification is a process by which the nutritional quality of food crops is improved through agronomic practices (Mitra-Ganguli et al., 2022). Biofortification differs from conventional fortification in that biofortification aims to increase nutrient levels in crops during plant growth (through the application of genetic engineering, breeding techniques, and other scientific methodologies) rather than through manual means during processing of the crops as defined by World Health Organization (WHO).

An illustration of biofortification's efficacy is Golden Rice, a variety engineered to be abundant in β-carotene, a precursor to vitamin A. The development and implementation of Golden Rice hold significant promise in addressing vitamin A deficiency in numerous countries where rice serves as a staple food (Stone & Glover, 2017).

Several studies, such as the work conducted by Carr and Vissers (Carr & Vissers, 2013), have demonstrated that the bioavailability of various nutrients, particularly AsA, is similar between dietary supplements and natural sources such as fruits and vegetables. However, the consumption of the latter is recommended due to their additional content of essential nutrients and health-enhancing phytochemicals. Considering these findings, biofortification can be perceived as a potential alternative to reliance on dietary supplements.

2.1.1. Ascorbic Acid in World Statistics

Ascorbic acid is an essential nutrient that must be consumed regularly to prevent deficiency. S. Rowe and A. Carr, in their comprehensive article titled "Global Vitamin C Status and Prevalence of Deficiency: A Cause for Concern?", analyze AsA data from diverse sources worldwide (Rowe & Carr, 2020). Based on the acquired data, the authors reached the conclusion that AsA deficiency is likely prevalent among low-income groups and low-middle-income countries. Illustratively, in North India, 74% of adults were observed to exhibit deficiency, while in South India, this percentage was found to be 46% (Ravindran et al., 2011). Similarly, research conducted in Russia indicated an exceedingly low average AsA status of 9 µmol/L, particularly among men, with high rates of deficiency (79%) and hypovitaminosis C (90%) among adult males (Paalanen et al., 2014). In the United States, based on the 2017-2018 surveys by the Centers for Disease Control and Prevention (CDC), the mean rate of AsA deficiency among adults (≥20 years) remained relatively stable at 6.75% compared to 6.99% observed in the 2005-2006 National Health and Nutrition Examination Survey (Powers et al., 2022). Furthermore, an estimated 25% of men and 16% of women within the low-income population of the United Kingdom exhibited plasma AsA concentrations indicative of deficiency (Mosdøl et al., 2008).

The existing recommended daily allowance (RDA) for AsA in the United States varies between 100-120 mg per day for adults (Naidu, 2003) which is equivalent to approximately two servings of baked potato (Pennington & Wilkening, 1997). Interestingly, AsA content documented in the USDA food database appears significantly lower, which however is not supported by literature (*FoodData Central*, 2019).

2.2. Role of Ascorbic Acid in a Human Body

Vitamin C, particularly its active form ascorbic acid, is a water-soluble micronutrient that cannot be synthesized by a human body. This section will primarily focus on its active form, aligning with our study's main focus.

AsA is engaged in a wide range of physiological functions, with one of its primary roles being the mitigation of oxidative stress. Oxidative stress is an imbalance between oxygen reactive species (ROS) and antioxidants. It causes cellular damage and is triggered by factors like UV, radiation, pollutants, and heavy metals. This stress contributes to age-related diseases and other health issues. AsA, a key antioxidant, curbs autoxidation by preventing free radical formation. This reduces oxidative stress, boosts immune function, and promotes healthy longevity (Carr & Maggini, 2017; Pizzino et al., 2017; Tan et al., 2018). The antioxidant activity of AsA has been associated with a potential reduction in cancer, diabetes, dementia, metabolic syndrome, osteoporosis, arthritis, and cardiovascular diseases incidences (Naidu, 2003; Padayatty et al., 2003; Wei et al., 2012).

The other primary function of AsA is that it serves as a cofactor in various biochemical processes, including the biosynthesis of signaling peptides like oxytocin, vasopressin, cholecystokinin, and calcitonin. It also plays a crucial role in collagen formation (Naidu, 2003), production of steroid hormones (Kobayashi et al., 2014) and influences DNA and histone methylation dynamics, potentially impacting embryonic development, aging, and anticancer therapies through epigenetic regulation (Blaszczak et al., 2019; Camarena & Wang, 2016; Paciolla et al., 2019).

In addition to its primary functions, AsA also participates in a multitude of other processes. It aids in the detoxification of excess histamine (Carr & Maggini, 2017), and supports the phagocytic functions of leukocytes. It plays a role in blood clotting (Bates et al., 1998) and tissue regeneration (Wei et al., 2012).

Interestingly, even though we commonly recognize ascorbic acid as a potent antioxidant, it can also paradoxically act as a pro-oxidant. Under conditions of high millimolar ascorbate concentration, AsA catalyzes the reduction of free transition metal ions, leading to the generation of oxygen radicals. This has the potential to disrupt the plant cell wall and trigger oxidative bursts (Green & Fry, 2005; Kaźmierczak-Barańska et al., 2020).

Deficiency in AsA can result in a range of adverse health conditions. These include anemia, scurvy, increased susceptibility to infections, muscle degeneration, impaired wound healing, the formation of atherosclerotic plaques, capillary hemorrhaging, elevated risk of myocardial infarction, and neurotic disorders (Fain, 2004; Hansen et al., 2014; Iqbal et al., 2004; NyyssÖnen et al., 1997).

As mentioned earlier, total vitamin C comprises the active form, ascorbic acid (AsA), and the inactive or oxidized form, dehydroascorbic acid (DHA). When AsA loses electrons in biosynthetic or antioxidant reactions, it transforms into the short-lived ascorbyl radical and then into DHA. In the human body, DHA can either be converted back to active AsA or metabolized further and participates in various reactions (Wilson, 2002).

2.3. Potato Nutritional Value and Agronomy

2.3.1. The Role of Potato in Global Food Security system

By the year 2050, it is projected that the global population will reach approximately 9.7 billion individuals. As per the Food and Agriculture Organization (FAO) report of 2009, this substantial increase in population is anticipated to lead to a surge in food demand, surpassing current consumption levels by more than 70% ((FAO), 2009). To effectively cater to the nutritional needs of this growing population, systematic advancements in agronomy and related industries are imperative. These improvements should be directed towards optimizing production efficiency within limited spatial constraints while concurrently mitigating the environmental impact. In achieving this, it becomes crucial to prioritize the utilization of crop varieties that exhibit the potential for high yield and enhanced nutritional composition. Such endeavors are vital for ensuring food security and sustainable development (Foley et al., 2011).

The potato is considered a crop that fulfills the above criteria. This crop demonstrates remarkable adaptability to changing environmental conditions, exhibits high yield potential, and serves as a valuable source of various macro- and micronutrients. Additionally, the potato has a longstanding history of playing a pivotal role in addressing food insecurities (Campos & Ortiz, 2019). Potatoes stand out by offering greater food production per unit of water compared to other major crops and exhibiting water use efficiency that is higher than cereals (Monneveux et al., 2013). Currently, the potato holds a significant position as one of the world's most important food crops in terms of human consumption, ranking closely behind wheat, rice, and maize. Cultivated across an estimated 20 million hectares of farmland globally, the potato boasts a remarkable worldwide production of 366 million tons (Devaux et al., 2021).

Global potato consumption statistics reveal that a substantial population of approximately 1.3 billion individuals rely on potatoes as a staple food, consuming more than 50 kg per person per year. Notably, regions such as India and China demonstrate a significant reliance on potatoes for their dietary needs (Campos & Ortiz, 2019).

The potato, as a crop, not only fulfills the requirements of the current food security system but also exhibits considerable potential for further improvement in relevant traits. This includes advancements in areas such as increased yields, enhanced resilience to climate change, improved resistance against diseases, and the potential for biofortification to enhance its nutritional composition. These future advancements hold the promise of bolstering the potato's contribution to food security and nutritional well-being.

2.3.2. Botany

Common potato (*Solanum tuberosum L.*) is a heterozygous and autotetraploid crop, with 2n = 4x = 48 chromosomes. It is a clonal crop that has undergone extensive breeding efforts, involving the hybridization of various cultivar groups and wild species, resulting in the development of modern varieties (Spooner et al., 2014). A cultivar, in the context of potatoes, refers to a cultivated variety that has originated naturally or through deliberate hybridization or random mutations, while genotype represents a variety, or variant of common potato with genetic differences, usually at the allelic level and is a portion of the genetic make-up of the accession or population. It possesses the ability to be reproduced either vegetatively, ensuring the propagation of identical plants (Burke, 2017). Potato market class is a comprehensive classification method that categorizes varieties (genotypes) and cultivars based on shared attributes like skin color, flesh color, shape, size, and suggested culinary applications. The Andes region in South America, where this crop originates, harbors a diverse range of potato varieties, with over 4,000 edible varieties documented (Campos & Ortiz, 2019). In the United States, there are more than 200 potato varieties utilized commercially across different sectors.

The tubers, the only edible part of the potato plant, are below-ground modified stems that are a storage organ for vegetative propagation. The tuber's anatomy includes distinct regions: the periderm or skin, which varies in thickness across varieties due to genetic and environmental factors (*Idaho Potato Comission*); cortex rich in starch; vascular tissue for nutrient transport; and medulla, a primary storage area. Nutrient distribution varies within tubers, with limited studies addressing this. For instance, Bandana S. and Kaushik S.'s research demonstrated higher dry matter, sucrose, and starch content in the cortex compared to the medulla (Bandana et al., 2016).

2.3.3. Potato Role in Human Nutrition

Potato has contributed to the human diet for thousands of years, initially in the Andes region of South America and then across the globe. The tubers of this plant are a source of various macro and micronutrients. It contains sugars, proteins, starches, lipids, phenolics, flavonoids, polyamines, carotenoids, and anthocyanins. Additionally, potatoes provide vitamins such as C, B1, B2, B3, B6, and K, as well as minerals including P, K, Ca, Fe, Zn, Mg, Mn, and Na. (Ezekiel et al., 2013). Due to its rich nutrient composition and low fat content, potato is considered a highly desirable component of the human diet (Visvanathan et al., 2016).

The extent of potato's contribution to the human diet is influenced by various factors, such as cooking methods, intake quantity, for example. However, it should be noted, that the initial (post-harvest) amount of minerals, vitamins, proteins, and dietary fiber does not always reach the consumer due the numerous factors affecting nutrient loss, which will be discussed in detail in the subsequent sections of this chapter.

As previously discussed, the extent of potato's contribution to the human diet is influenced by the level of potato intake. In certain developing nations, potato average daily consumption can range from 50 to 150 g for adults. Conversely, in low-income countries, rural areas of Africa, and highland regions of Latin American countries, potatoes are regarded as a staple crop and are consumed in significant quantities, ranging from 300 to 800 g per day for adults (Campos & Ortiz, 2019).

2.3.4. Ascorbic Acid in Potato

The widespread availability of potatoes in countries with different income levels ensures that they serve as a reliable source of various nutrients, including daily intake of AsA. The specific content of this vitamin in potato tubers can vary depending on multiple factors and conditions. Based on available publications and data from the United States Department of Agriculture (USDA), it can be generally observed that freshly harvested potatoes can have a AsA content that exceeds 40 mg/100 g FW, with a minimum value of approximately 10 mg/100 g FW. Moreover, in some studies yellow and russet genotypes have higher AsA levels than red and specialty (Külen et al., 2013). It is important to note that significant variations in AsA concentrations occur due to factors such as genotype, environment, genotype by environment interactions, and other relevant factors (Burgos et al., 2009; Dale et al., 2003; Thomas et al., 2021). These factors will be further explored and discussed in detail in the present study.

AsA is known for its health benefits in humans, but it also plays a significant role in plants. It acts as an antioxidant, directly scavenging reactive oxygen species (ROS) and removing hydrogen peroxide (Asada, 1999; Davey et al., 2000). Additionally, it is involved in cell division and programmed cell death (PCD) in plants (Liso et al., 1984). It also is as a natural inhibitor of enzymatic browning in potatoes (Rocculi et al., 2007; Yildiz, 2019) and participates in iron uptake

(Lane & Richardson, 2014). This characteristic presents an advantage for the processing industry, as it has the potential to reduce losses associated with enzymatic browning. AsA in plants also serves as a cofactor for 1-aminocyclopropane-1-carboxylic acid (ACC) oxidase, which is essential for ethylene synthesis (Murphy et al., 2014). Furthermore, its role as a cofactor for dioxygenases suggests its potential involvement in the synthesis of abscisic acid and gibberellins, as well as the breakdown of auxins in plants (Smirnoff, 2018).

Potatoes, as a staple crop, provide significant AsA in human diets despite the variation in the initial content and potential loss. Enhancing AsA in potatoes through breeding and improved practices is viable (Love & Pavek, 2008) and biofortified crops offer a global solution for AsA deficiency, benefiting human health and enhancing plant stress tolerance amid climate change challenges (Paciolla et al., 2019).

2.4. Factors Affecting Ascorbic Acid Level in Potato

2.4.1. Ascorbic Acid Accumulation in Potato

AsA accumulation in plants depends on several metabolic processes, including biosynthesis, recycling, degradation, and transport. There are four primary biosynthetic pathways described that contribute to AsA production in plants: the D-mannose/L-galactose, gulose, myoinositol, and galacturonate pathways (Paciolla et al., 2019). Whereas D-mannose/L-galactose pathway, also known as Smirnoff-Wheeler pathway is considered to be the primary one in higher plants (Fig. 2.1) (Mellidou et al., 2012).

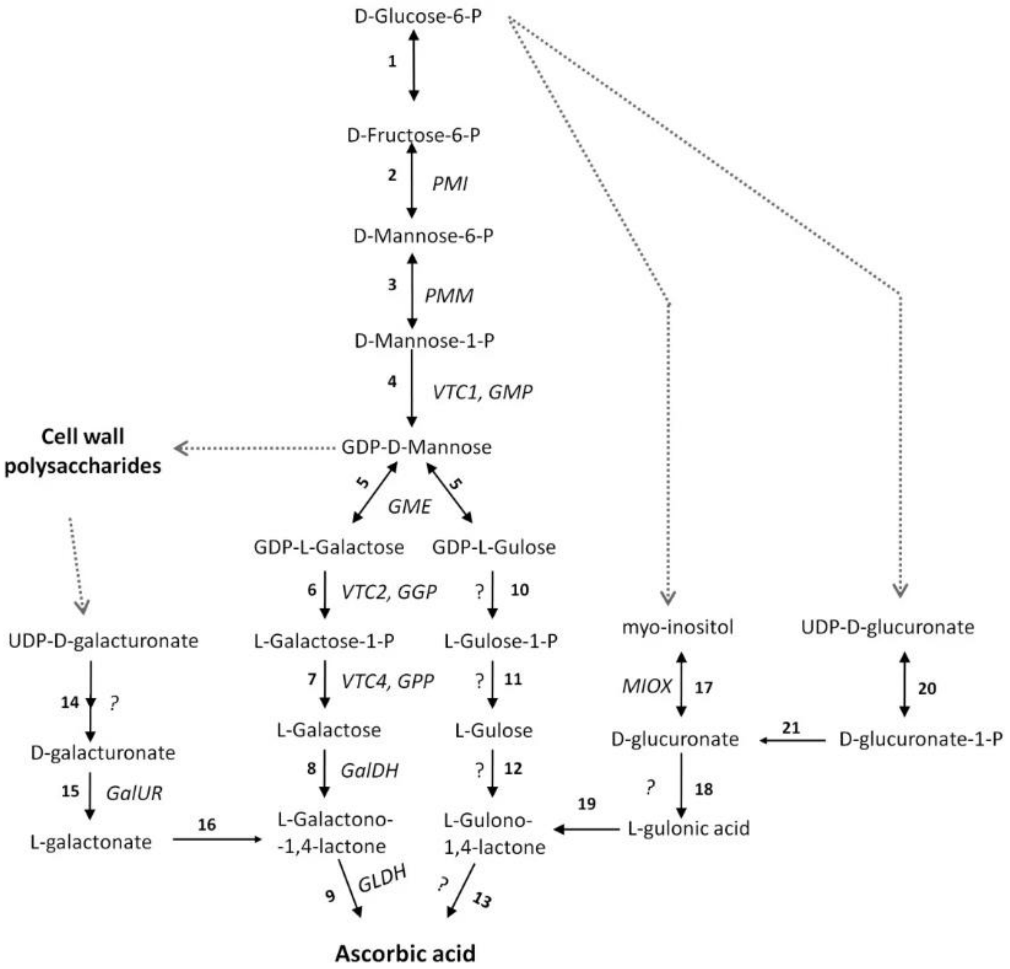

Figure 2.1: The main (primary) and the alternative AsA biosynthetic pathways in higher plants.

Reprinted from "Regulation of fruit ascorbic acid concentrations during ripening in high and low vitamin C tomato cultivars," by Ifigeneia Mellidou, Johan Keulemans, Angelos K Kanellis & Mark W Davey, 2012, BMC Plant Biology (Mellidou et al., 2012).

Some studies revealed active AsA synthesis in cut potato tubers in response to injury (Johnson & Schaal, 1957; Ôba et al., 1994). More recent research by Tedone et al. (2004) indicated that AsA synthesized in leaves can be transported to developing tubers suggesting a link between foliar and tuber AsA levels, but this hypothesis remains unexplored (Tedone et al., 2004). Also, multiple publications suggest that the AsA content in potatoes is influenced by the presence of light at certain growing stages. Specifically, exposure to intense light has been found to increase vitamin AsA, while the absence of light, can lead to a decrease in its levels (Fukunaga et al., 2010; Gatzek et al., 2002; Seminario et al., 2017; Wheeler et al., 1998).

A thorough understanding of the regulatory mechanisms for ascorbic acid accumulation is essential for enhancing biofortification efficiency, considering variations among crops and growth stages, and selecting more effective techniques.

2.4.2. Mechanisms of Ascorbic Acid Retention and Degradation in Potato Tubers

Nutrient retention refers to the quantity or proportion of a nutrient that remains in a food after undergoing changes, such as cooking or exposure to various factors, compared to the original nutrient content in the food (Murphy et al., 1975). AsA, being a water-soluble and unstable compound, is susceptible to degradation caused by light, heat and oxygen (McGill et al., 2013).

In most plants, ascorbic acid (AsA) degradation occurs via dehydroascorbic acid (DHA), which can be further oxidized or hydrolyzed to form diketogulonate (DKG), resulting in a permanent loss of AsA. The initial oxidation step between AsA and DHA can be reversible in plants due to the presence of DHA reductase (Dewhirst et al., 2017; Green & Fry, 2005).

Typically, DHA accounts for less than 10% of the total vitamin C content in potato tubers. This ratio has led researchers to either measure the combined content of both components by reducing DHA to AsA, a process that may occur naturally in the human body, or focus solely on AsA when assessing vitamin C levels in potatoes (Hamouz et al., 2018; Tedone et al., 2004; Valcarcel et al., 2015a). The latter approach was implemented in our study. In some publications referenced in this thesis, the terms "vitamin C," "ascorbic acid," and "ascorbate" were used interchangeably.

The tuber remains a living tissue post-harvest, exhibiting ongoing reactions that can modify its metabolic profile. This was supported by Datir et al.(2020), study, alterations in sugars, sugar alcohols, amino acids, and organic acids are evident in selected varieties after 1 month of cold storage compared to harvest (Datir et al., 2020). Another publication focusing on room temperature, dark storage at intervals of 1, 5, 8, 11, and 15 weeks after harvest also highlights substantial variations in specific metabolites at each time point. Collectively, these studies emphasize that the tuber maintains its vitality as a living tissue, undergoing changes under diverse storage conditions (Uri et al., 2014).

In Summary, due to the instability ascorbic acid and abundance of factors affecting it, it is important to try to preserve its level in potato tubers for its maximum benefit for human health. Therefore, scientists and producers are constantly in the process of developing methods and approaches aimed at both improving the initial content of nutrients in the product and their effective preservation.

As mentioned above, AsA content in potato tubers varies based on market class and genotype. However, numerous pre- and post-harvest factors, including but not limited to growing conditions, genetics, storage conditions and duration, additional processing, and cooking, can also impact its content.

Multiple studies conducted in various European countries have reported a positive association between higher AsA content in potato tubers and drier climatic conditions (Hamouz et al., 2007; Valcarcel et al., 2015a; Valcarcel et al., 2015b). Similar data were acquired in a study in Ireland where lower rainfall demonstrated a correlation with the higher AsA content in tubers (Hamouz et al., 2018). However, another publication showed no effect of drought on AsA content except for one genotype (Andre et al., 2009). Additionally, a study has suggested that lower minimum daily temperatures during the vegetative period and higher altitude may contribute to higher AsA content (Lachman et al., 2008). Despite the demonstrated dependence of vitamin AsA on environmental factors, the authors of these studies acknowledge the significance of other factors unique to the crop year, such as stress factors, mechanical damage to the tubers, and pathogens presence.

As was noted before the initial level of AsA accumulates in potato through the Smirnoff-Wheeler pathway and depends on the specific genotype (cultivar). Thus, the genotype x environment interaction (GxE) reflects the different responses of certain cultivars to changing environmental conditions (Rymuza & Bombik, 2010) or it can be described as when external stimuli in a form of agroclimatic conditions modulate gene expression of AsA. In multiple articles, this interaction is proven to be significant for different potato characteristics including AsA (Dale et al., 2003; Thomas et al., 2021).

In some articles testing the same cultivars and genotypes over different locations, significant variation in AsA content due to genotype, environment, and genotype × environment interaction was found (Burgos et al., 2009; Love et al., 2004).

As it was shown in multiple studies, potato tubers have characteristics that allow them to be stored for extended periods. However, as storage time increases, undesirable changes occur in the quality and chemical composition of the tubers (Keijbets & Ebbenhorst-Seller, 1990; Lee & Kader, 2000; LEŠKOVÁ, 2006; Yamdeu Galani et al., 2017).

Thus, according to Burgos et al., AsA retention levels after storing under farming conditions have been shown to vary between 22 and 62%, depending on the variety (Burgos et al. 2009). Similar results were demonstrated in another publication with the emphasis on the fact that AsA content decline was not gradual and the most rapid losses happened within 2-3 months of storage with a slower decrease after (Külen et al., 2013).

Storage temperature is another aspect along with duration, which affects the nutrient composition of potatoes. Many studies show a decrease in AsA levels with cold storage for different varieties (Goyer et al., 2019; Keijbets & Ebbenhorst-Seller, 1990; Külen et al., 2013; Oktay, 2013). However, in another study, 15 Indian potato varieties were reported to demonstrate an increase in AsA levels during 90 days of room-temperature storage (Yamdeu Galani et al., 2017). Additionally, it's worth noting that metabolic processes slow down when potatoes are stored at lower temperatures and during dormancy. However, as potatoes enter the sprouting phase, metabolism intensifies. During sprouting, stored nutrients in the tuber, including ascorbic acid, are utilized to support the development of new shoots and foliage (Espen et al., 1999).

Numerous studies have investigated various cooking methods to determine their efficacy in minimizing nutrient loss. Overall, key nutrients such as minerals, proteins, and dietary fiber in potatoes are generally well retained after cooking (Burgos et al., 2009). However, vitamins C content demonstrates substantial reduction after cooking with the degree of loss influenced by the specific cooking method employed. Losses occur through leaching into the cooking water, heat-induced degradation, and oxidation. For instance, the boiling of peeled potatoes has been observed to increase losses by approximately 10% compared to unpeeled tubers, where the skin acts as a protective barrier against leaching (Woolfe & Poats, 1987).

Several studies have established a negative relationship between cooking temperature and duration and the concentration of AsA in potatoes, indicating that employing lower cooking temperatures and shorter cooking times can enhance nutrient bioavailability (Han et al., 2004; Jayanty et al., 2019). Multiple studies show microwaving as the least destructive, compared with backing, boiling, and frying (Buresh et al., 2021; Stushnoff et al., 2008; Thomas et al., 2021).

2.5. Summary and Research Objectives

2.5.1. Summary

Potato, as a staple crop in numerous countries and known for its health-promoting properties, holds significant potential for enhancement and fortification considering the increasing global population and shifting environmental conditions. With its considerable AsA content, potato can serve as a primary or one of the primary sources of this essential nutrient required for daily human consumption.

2.5.2. Objectives

The primary objective of this study was to improve our understanding of the potential to breed for enhanced AsA C content and AsA retention. This objective was investigated by measuring impacts of genetics and environment on AsA dynamics from harvest to storage, and specifically within a breeding population and across several growing locations. The specific objectives of the study were as follows:

1. Determine variation in tuber AsA concentration, among 34 genotypes in a breeding population.
2. Determine variation in AsA retention during storage, among 34 genotypes in a breeding population.
3. Determine the effect of genotype and environment interaction on AsA content and retention for a sub-set of genotypes grown in three locations.

2.5.3. Hypotheses

I hypothesize that there is variation among genotypes in postharvest AsA content and retention during storage time.

I hypothesize there is a variation among genotypes grown in different locations in AsA content and retention due to genotype and environment interaction.

CHAPTER 3 - STUDY 1. THE EFFECT OF GENOTYPE ON ASCORBIC ACID CONTENT AND RETENTION DURING STORAGE

3.1. Introduction

In general, the potato tuber achieves maximum AsA content directly after harvest, and this is reduced during long-term cold storage that is a regular process in the potato industry. Most potatoes are stored for up to 12 months prior to consumption. It therefore is important to understand the fundamentals of AsA retention in tubers in the context of storage, specifically by modeling its dynamics that occur over the course of several months. Understanding degradation of AsA and the genotypic and environmental factors that influence these rates can lead to developing strategies to preserve as much of the AsA content as possible. This can be potentially achieved through implementing specific storage conditions or/and selecting genotypes that exhibit better retention of AsA over extended storage periods. By adopting such measures, it will help to maintain the nutritional value of potatoes. Therefore, it is essential to investigate the variation in degradation rates among different genotypes during storage.

3.2. Objectives

The primary objectives of the first year of this study were to determine variation in tuber AsA concentration and its retention attributed to genotype, among 34 genotypes in a breeding population obtained from the San Luis Valley Research Center in Colorado, CO.

3.3. Materials and Methods

3.3.1. San Luis Valley Environmental and Growing Conditions

Plant materials for Year 1 study were provided by the San Luis Valley Research Center (SLVRC), Center, Colorado, USA in 2021. At the SLVRC, potatoes were grown under cool temperatures (95 frost-free days), with average highs ranging from 80-90F (27-35°C). Planting season typically starts in late spring (mid-April or May) in the area, depending on weather and soil conditions. Harvesting occurs in fall. The growing site situated at approximately 2,300 meters (7,600 ft.) above sea level, offers unique growing conditions for potatoes due to the high altitude and cool nights. Water availability is crucial and (is a limitation factor) in the San Luis Valley, thus, irrigation playing a vital role in maintaining adequate moisture. The SLVRC experiences an average precipitation level of approximately 2.5-5 cm from May to September, subject to yearly variations. There were few instances of hail throughout the growing season.

3.3.2. Storage Conditions

All tubers were harvested from the field plots when they reached maturity, approximately two weeks after the vine kill from field plots using conventional agronomic methods in September and stored at 4°C, in the dark. At the beginning of October, they were transported to Fort Collins, CO, USA for storage and the following analysis. Storage conditions remained the same.

3.3.3. Plant Material

The genotypes used in this study were selected to represent a diverse set of breeding material, covering a spectrum of expected high, medium, and low AsA content. The selection process was based on extensive data spanning 20 years, obtained from the United States Department of Agriculture (USDA), and analyzed. All genotypes provided by San Luis Valley Research Center (SLVRC), Colorado are listed in the Table 3.1.

Table 3.1: Genotypes and characteristics of 34 selected potato genotypes grown at SLVRC in 2021.

Genotype	Skin Color	Flesh Color	Market Class
AC06908-1W/Y	White	Yellow	Yellow
AC08172-2W/Y	White	Yellow	Yellow
AC11436-1RU	Russet	White	Russet
AC11573-3R/Y	Red	Yellow	Specialty/Red
C220-1	White	Yellow	Yellow
CO07044-3RU/Y	Russet	Yellow	Specialty/Russet
CO10064-1W/Y	White	Yellow	Yellow
CO10085-1RU	Russet	White	Russet
CO10098-5W/Y	White	Yellow	Yellow
CO11009-3RU	Russet	White	Russet
CO11250-1W/Y	White	Yellow	Yellow
CO11266-1W/Y	White	Yellow	Yellow
CO12378-1RU	Russet	White	Russet
CO13029-2RU/Y	Russet	Yellow	Specialty/Russet
CO13188-2RU/Y	Russet	Yellow (Orange)	Specialty/Russet
CO14206-1W/Y	White	Yellow	Yellow
CO14226-3W/Y	White	Yellow	Yellow
CO14247-2W/Y	White	Yellow	Yellow
CO14371-3RU	Russet	White	Russet
CO15016-1RUsto	Russet	White	Russet
CO15084-2R	Red	White	Red
CO15206-7R	Red	White	Red
CO15211-1R	Red	White	Red
OR04198-1	Yellow	Yellow	Yellow
POR11PG62-3	Yellow/Red	Yellow	Specialty/Yellow
Castle Russet	Russet	White	Russet
Criolla 8	Yellow	Yellow	Specialty/Yellow
Fortress Russet	Russet	White	Russet
Inka Gold	White/Purple	Yellow	Specialty/Yellow
Ranger Russet	Russet	White	Russet
Rio Grande Russet	Russet	White	Russet
Russet Burbank	Russet	White	Russet
Sangre-S10	Red	White	Red
Yukon Gold	White	Yellow	Yellow

3.3.4. Sampling and Processing

Randomly selected sets of five tubers from each genotype were collected at each storage sampling time point (every two weeks), ensuring that unusually large or small tubers were avoided.

A sampling schedule was established, consisting of four sampling dates spaced at intervals of two weeks. The selected sampling dates are as follows: November 11, 2021; November 25, 2021; December 9, 2021; and December 23, 2021.

For each genotype, the five tubers were washed in tap water, air dried, weighed, and placed into sealed plastic bags at -80°C to stop any further AsA degradation. Once frozen, the five tubers from each genotype underwent mechanical crushing using a hammer and returned to a temperature of -80°C for stabilization prior to freeze-drying using a Harvest Right freeze-drier. The resulting dried samples were weighed, ground using a coffee grinder, and stored in plastic bags at -20°C until further chemical analysis.

3.3.5 Chemical Analysis

The extraction of ascorbic acid from potato samples was carried out using aqueous solution of metaphosphoric acid (30 g/L) and acetic acid (80 g/L). The extraction procedure was conducted as follows: In a 15 mL centrifuge tube, 12.5 mL of the extraction solution was added to 83.3 mg of the dry and ground potato sample. The mixture was then subjected to vortexing at room temperature for a duration of 20 min to ensure proper homogenization. Subsequently, the tube was centrifuged at 6,000 RCF for 20 min at 10 C, and 1-2 mL of the resulting supernatant was filtered using syringe filters (Syringe Filters, PVDF, Sterile, 0.2 µm) into glass HPLC vials.

For the preparation of ascorbic acid standards, 10 mg of L-ascorbic acid (Sigma-Aldrich Corporation, St. Louis, MO) was mixed with 10 mL of the extraction solution, and subsequent dilutions (20, 10, 5, 2.5, and 1.25 µg) were made to generate a five-point standard curve.

The determination of AsA content was performed using high-performance liquid chromatography on PerkinElmer LC 300 equipped with a PDA detector (PerkinElmer Inc.). Isocratic elution of ascorbic acid was achieved using a mobile phase consisting of 0.03M sodium acetate in a 5% methanol solution (pH 5.8) at a flow rate of 1 mL/min. Absorbance readings were taken at 254 nm, and the expected retention time for AsA was approximately 2.35 min. Samples were injected onto a Waters XSelect (HSS C18 SB 3.5 µm, 4.6 x 150 nm) column (Waters Corporation, Milford, MA). The obtained results were processed using Simplicity software and expressed in terms of milligrams per 100 grams of fresh matter or fresh weight (mg/100 g FW).

3.3.6. Statistical Analysis

To analyze the data acquired from the HPLC analysis, a comparison of the slopes was conducted among the 34 potato genotypes. Slopes were calculated in Excel using the SLOPE formula. AsA Loss in % was calculated in Excel using formula:

$$\frac{(Initial\ AsA - Final\ AsA)}{Initial\ AsA * 100}$$

The variations in slopes can provide insights into the rate of degradation or retention of AsA in each variety. Additionally, a heat map was performed using RStudio (v. 0-421, Integrated Development Environment for R, Boston, MA) to visually depict the degradation of AsA for each variety over time using a color gradient, thereby facilitating the clear presentation of the results. The Heatmap is accompanied by a dendrogram to better understand similarities and differences between AsA levels in selected potato genotypes. Both the heat map with the dendrogram and summary table are presented below (Figure 3.1, Table 3.2). Comparing slopes of different potato genotypes allows us to evaluate the rate at which the AsA content is changing over time. This comparison can provide valuable information for further analysis and interpretation.

3.4. Results

AsA content was measured for all 34 genotypes across four time points, with Time 1 expected to have the highest AsA content and Times 2-4 (56, 70, and 84 days) representing longer storage periods with expected reductions in content. The full experiment results were expressed as a heat map to observe trends in the data (Figure 3.1). Up to four-fold differences in AsA content were observed at Time 1.

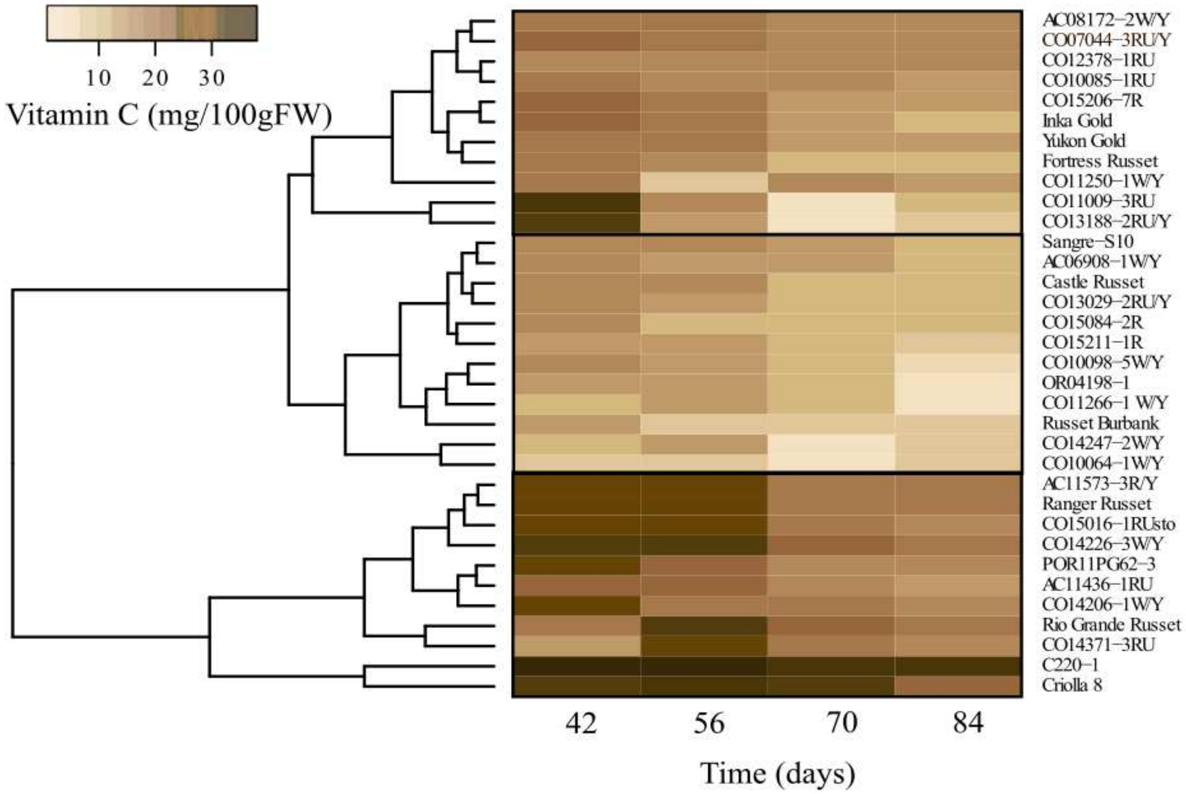

Figure 3.1: Heatmap with dendrogram showing AsA degradation of 34 selected genotypes during storage.

Based on dendrogram analysis, three distinct major clusters were identified. The first major cluster of genotypes (at the top) showed steady, roughly linear declines in AsA content across the four time points. Notably, there was a subcluster at the bottom with two genotypes (CO11009-

3RU, CO13188-2RU/Y) with excessively high-to-low shifts, specifically a drop in AsA levels at Time 3 followed by an increase to some extent, but not to the previous level. Another interesting pattern was shown by genotype CO11250-1W/Y, which exhibited a similar pattern, but the drop occurred earlier at Time 2, followed by a subsequent increase to the initial level. Data from more years or other research is needed to check the consistency or prove the error.

The second cluster, or the middle cluster, started with generally low AsA content and remained low, with the most noticeable decline between Times 2 and 3 for most of the genotypes. If we look at the last two genotypes in this cluster (CO14247-2W/Y, CO10064-W/Y), we can notice a similar pattern to the last two from the first cluster. Supposedly, differences in initial AsA content didn't allow them to be placed in the same cluster.

The third cluster, or the bottom cluster, exhibited high initial levels and generally maintained this high profile throughout storage. Similarly, to the second cluster, the third cluster showed the most significant loss occurring between Times 2 and 3. Among the genotypes in the third cluster, C220-1 and Criolla 8 stood out for their high initial and final AsA content, which demonstrated minimal degradation.

The heat map with the dendrogram supports that all 34 genotypes demonstrated some level of AsA degradation during storage but indicates variation in retention patterns, as shown through the clusters. No trends based on market class, skin color, or flesh color could be identified. Further investigation is required for genotypes exhibiting anomalous patterns, such as an increase in AsA content in the middle of storage or other fluctuations. To complement the heatmap coupled with the dendrogram, which provides visual insight into patterns of AsA degradation across genotypes, calculated loss (%) and slopes are presented in Table 3.2.

Table 3.2: Summary table of AsA content[a] of 34 selected genotypes grown at the San Luis Valley Research Center, Center, Colorado in 2021.

Genotype	**Market Class[b]**	**Initial AsA Content**	**Final AsA Content**	**Mean**	**Slope**	**AsA Loss[b] (%)**	**Cluster**
AC06908-1W/Y	Y	15.8	10.3	13.6	-0.1283	34.6	2
AC08172-2W/Y	Y	19.6	15.3	17.9	-0.1134	21.6	1
AC11436-1RU	RU	23.6	14.8	19.2	-0.2198	37.2	3
AC11573-3R/Y	S/R	25.1	19.5	22.3	-0.1415	22.0	3
C220-1	Y	37.7	32.0	34.3	-0.1806	15.0	3
CO07044-3RU/Y	S/RU	21.7	15.3	17.9	-0.1559	29.5	1
CO10064-1W/Y	Y	8.5	7.6	6.3	-0.0729	11.0	2
CO10085-1RU	RU	18.3	13.9	16.4	-0.1044	23.9	1
CO10098-5W/Y	Y	16.7	4.5	11.5	-0.2714	73.0	2
CO11009-3RU	RU	29.9	11.9	15.3	-0.5051	60.4	1
CO11250-1W/Y	Y	19.7	14.4	14.8	-0.0497	26.7	1
CO11266-1 W/Y	Y	11.8	2.2	9.4	-0.2279	81.0	2
CO12378-1RU	RU	17.6	15.2	16.7	-0.0577	13.4	1
CO13029-2RU/Y	S/RU	15.6	11.9	13.4	-0.1061	23.9	2
CO13188-2RU/Y	S/RU	28.2	7.2	12.5	-0.5233	74.4	1
CO14206-1W/Y	Y	25.0	15.9	20.5	-0.1953	36.5	3
CO14226-3W/Y	Y	27.8	19.1	23.9	-0.2270	31.3	3
CO14247-2W/Y	Y	11.8	8.5	8.9	-0.1515	28.6	2
CO14371-3RU	RU	13.1	17.1	18.6	0.0422	-31.1	3
CO15016-1RUsto	RU	25.6	16.5	21.2	-0.2299	35.7	3
CO15084-2R	R	16.7	11.9	13.1	-0.1080	29.1	2
CO15206-7R	R	23.0	13.4	17.5	-0.2403	41.8	1
CO15211-1R	R	13.8	9.2	11.7	-0.1204	33.4	2
OR04198-1	Y	14.9	2.6	10.9	-0.2845	82.4	2
POR11PG62-3	S/Y	24.2	16.1	20.1	-0.2103	33.5	3
Castle Russet	RU	15.4	10.2	13.5	-0.1420	33.8	2
Criolla 8	S/Y	27.6	23.1	27.3	-0.1300	16.1	3
Fortress Russet	RU	19.5	11.0	14.8	-0.2123	43.6	1
Inka Gold	S/Y	23.1	11.8	17.9	-0.1109	48.8	1
Ranger Russet	RU	25.0	19.2	22.0	-0.1659	23.3	3
Rio Grande Russet	RU	28.0	20.0	23.2	-0.2879	28.7	2
Russet Burbank	RU	13.3	7.1	9.5	-0.1385	46.4	2
Sangre-S10	R	17.6	10.0	14.2	-0.1759	43.4	2
Yukon Gold	Y	19.1	13.1	16.3	-0.1651	31.3	1
Mean		20.4	13.3	16.7	-0.1798	34.8	
SD		6.3	5.8	5.6	0.1088	21.5	
Mean Loss Per Day[a] = 0.157							

[a] mg/100 g FW.

[b] RU = Russet, R = Red, Y = Yellow, S/RU = Specialty/Russet, S/R = Specialty/Red, S/Y = Specialty/Yellow.

As demonstrated in Table 3.2, the mean loss across all 34 genotypes was 34.8%. The collective daily average loss for all genotypes amounted to 0.157 mg/100 g FW. Interestingly, one genotype, CO14371-3RU, exhibited an unexpected increase in AsA content, an occurrence that has not been previously documented in existing studies. The AsA loss data presented in Table 3.2 has been utilized to generate Figure 3.2, which provides an overview of the distribution of AsA loss among the six studied market classes after the storage period. In terms of market class-specific loss, specialty/red potatoes showed the smallest loss, at 22%. However, it's important to note that this class was represented by only one genotype (AC11573-3R/Y). Thus, no error bars are applicable in this case. The specialty/Russet market class showed the highest loss of 42.6%. In general, high variation is observed within each market class, as demonstrated by standard deviation (SD) error bars.

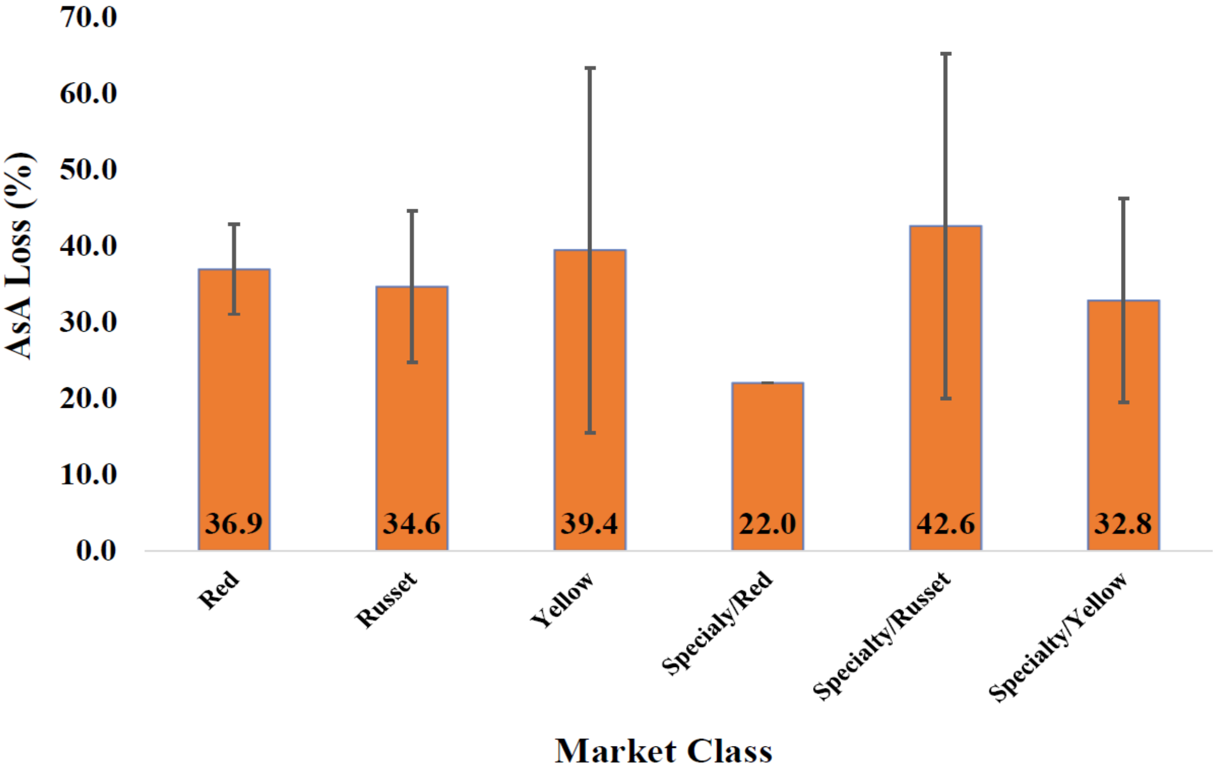

Figure 3.2: Mean AsA loss (%) among different market classes.

3.5. Discussion

The general AsA loss was observed in most of the genotypes is substantial, with multiple publications supporting this fact (Goyer et al., 2019; Külen et al., 2013). Variation in AsA retention was also observed in these genotypes, and this finding is supported by several publications. Interestingly, certain genotypes displayed a temporary drop in AsA between Times 2 and 3, followed by an increase to some extent. This pattern was not supported in the literature and should be investigated further.

Several genotypes exhibited a temporary increase in AsA content in the middle of the storage period, and this fluctuation was partially supported by prior findings (Yamdeu Galani et al., 2017). In their study, this increase was observed after 30 days of storage at 4°C, with some genotypes displaying higher AsA levels than their initial values by the end of the storage period. However, there was no overlap in genotypes between our study and theirs. Fluctuations in AsA can also be linked to the end of dormancy and the beginning of sprouting period, during which stored nutrients in the tuber, including AsA, are utilized to support the growth of new shoots and leaves (Espen et al., 1999).

Two genotypes, C220-1 and Criolla 8, consistently exhibited high AsA levels from the beginning to the end of the storage period, showing minimal degradation. As a result, they may be strong candidates for breeding, especially if they possess other desirable qualities.

In Chapter 2, we explored the various factors influencing AsA content and degradation in potato tubers. Based on the prior discussions and the results obtained in this chapter, an interesting area to investigate is whether market class can serve as a simpler means to predict AsA (and perhaps other nutrient) trends. However, it should be noted that our study was limited to only 34 genotypes from a specific location, which may not represent the entire diversity of potato genotypes. Therefore, more research is needed to understand the relationship between market class and AsA loss in a broader range of potato genotypes. Market class, as a multifaceted variable for grouping genotypes, introduces physiological, chemical, and morphological variability within a given class, such as skin thickness, shape, and size, thereby adding several subfactors contributing to AsA variation. For instance, the possibility of thicker skin in russet potatoes could serve as a factor that may mitigate oxidation during storage.

Additionally, understanding the metabolomics of potato tubers including the presence of other antioxidants or AsA precursors after harvest may shed light on how they influence AsA retention during storage. This area needs further investigation to determine if other compounds play a significant role in maintaining AsA levels.

In conclusion, more research is needed to fully understand the relationship between market class, potato genotype, and AsA dynamics. Exploring these aspects can help optimize storage methods and preserve the nutritional value of potatoes. The results obtained this year were derived from genotypes cultivated in a single growing location. Existing publications (Burgos et al., 2009; Thomas et al., 2021) have demonstrated that location (environment) or genotype-environment interaction can significantly influence the composition of potato metabolites. Despite this potential complexity, we made the decision to proceed with the study to investigate the effects of these factors on potato metabolites, including AsA content.

3.6. Conclusion

In conclusion, the results from our study provides additional supporting information on the potential to breed for potato varieties with increased AsA, and specifically adds new knowledge on genotypic effects on AsA retention during storage by exploring AsA content for broad number of genotypes. Notably, there was considerable variation in AsA degradation rates and patterns among the genotypes, with a few even demonstrating a temporary increase in AsA content in the middle of the storage period.

The observed variations in AsA degradation rates may be attributed to a combination of genetic factors and environmental influences. To gain a more comprehensive understanding of the underlying mechanisms, it is essential to integrate environmental factors, such as growing locations, in the Year 2 study. This approach will facilitate the investigation of genotype-environment interactions and their implications for AsA retention in potato tubers. By considering both genetic and environmental aspects, we can further unravel the complexities of AsA dynamics and develop strategies to optimize AsA retention during storage.

CHAPTER 4 – STUDY 2. THE EFFECT OF ENVIRONMENT ON ASCORBIC ACID CONTENT AND RETENTION DURING STORAGE

4.1. Introduction

Based on the findings from the Study 1, previous published studies, and data obtained from the Western Regional Trial dataset spanning from 1997 to 2021, a selection of six genotypes exhibiting varying levels of AsA content (three low and three high) was identified for further investigation in the Year 2 study conducted in 2022. The Year 2 study aimed to examine the potential interaction between genotype and environment on post-harvest AsA content and degradation rate in tubers. To address this, three locations with differing environmental conditions were selected to conduct the study. In this study, growing location serves as the representation of the environmental factor, thus "Location" will be interchangeably used with "Environment." This part of the study was carried out in collaboration with Texas A&M University and Oregon State University.

4.2. Objectives

The primary objectives of the second year of this study were to investigate and quantify the variation in initial AsA content, as well as its retention, among six selected genotypes grown in three locations and to explore the sources of these variations.

4.3. Study Design and Methods

A different approach was used for Year 2 study compared with Year 1. In this part of the study, the practice of combining every five tubers from each genotype at each time point was omitted thereby enabling the attainment of five biological replications in addition to technical replications.

4.3.1. San Luis Valley Growing Conditions

The growing conditions of the San Luis Valley location in Colorado were previously discussed in Chapter 3. However, notable differences were observed in the year 2022, including the absence of significant hail events and a slight elevation in the average temperature.

4.3.2. Oregon Site Growing Conditions

The plant material used in this study was obtained from Oregon State University's growing site in Hermiston, Oregon, situated at an altitude of 196 meters (643 ft.). The year 2022 was characterized by extreme heat throughout the growing season, particularly during the early season from June to August. The soil type at the site is fine sandy loam. The crop was planted in mid-April, with the harvest taking place at the end of September. For the late trials, irrigation and rainfall amounted to 85 cm (33.5 inches).

4.3.3. Texas Site Growing Conditions

Plant material from Texas was provided by the Texas A&M AgriLife Research Department of Horticultural Sciences at Texas A&M University. The research trials were conducted 10 miles southwest of Dalhart, Texas, USA, at an altitude of 973 meters (3,192 ft). The soil type at this location is Tivoli fine sand. The crop was planted at the beginning of May 2022, and the harvest occurred in mid to late September. Throughout the growing season, there was an instance of light hail, and the total recorded precipitation and irrigation amounted to 83.8 cm (33 inches).

4.3.4. Storage Conditions

Plant material from the three growing sites were received and stored in a controlled environment at a temperature of 4°C (39°F). To maintain optimal conditions, the entirety of the plant material was placed on ventilated shelves within their original packaging comprising cardboard boxes and bags utilized during shipment.

4.3.5. Plant Material

Based on the findings from the Year 1 study, previously published studies, and data obtained from the Western Regional Trial dataset spanning from 1997 to 2021, a selection of six genotypes exhibiting varying levels of AsA content (three low and three high) was identified for this part of study. The corresponding genotypes are presented in Table 3.1 in descending order of AsA content, ranging from high to low.

Table 4.1: Six potato entries, selected to be grown in three locations[a] for Year 2022 study and their AsA content based on Western Regional Trial[b] data.

Genotype	Years of Data	AsA content (mg/100 g FW[c])
Yukon Gold	22	38.6
Rio Grande Russet	3	35.6
Ranger Russet	23	32.0
CO10064-1W/Y	2	16.9
Sangre-S10	2	16.5
CO11266-1W/Y	2	15.8

[a] Locations: Colorado, Oregon, Texas.
[b] Data from Western Regional Trials (Idaho) 1997-2021.
[c] FW, fresh weight.

4.3.6. Sampling and Processing

Randomly selected samples consisting of five tubers from each genotype from each location were obtained for each storage time point (five time points total), ensuring that unusually large or small tubers were avoided. Subsequently, the samples were subjected to the same sample preparation method employed in the Year 1 study. However, a modification was made for this study: each of the five tubers from the same genotype, collected from the same location and sampling time point, was individually placed in a separate plastic bag. This approach ensured that the plant material was never combined, thereby enabling biological replications.

4.3.7. Chemical Analysis

The chemical analysis procedure remained consistent with the methodology outlined in section 3.4.6 of Chapter 3. To minimize experimental bias, all samples were assigned a random number prior to sampling and analysis.

4.3.8. Statistical Analysis

The data obtained from HPLC analysis were subjected to Analysis of Variance (ANOVA) and pairwise comparisons of slopes and intercepts to assess the significance of differences in AsA retention among genotypes and variations in AsA content among the same genotypes grown in different locations. Differences were considered significant if the p-value (P) was ≤ 0.05. Analysis of Variance and pairwise comparisons were conducted in RStudio (version 0-421). AsA % Loss was calculated in Excel using the formula from section 3.4.7 of Chapter 3.

4.4. Results

The results showed evidence of variation in AsA content among the different genotypes during storage. At the initial sampling time point, the range of content was from 15.4 mg/100 g FW (CO10064-1W/Y , CO) to 40.0 mg/ 100 g FW (Rio Grande Russet, TX). At the last time point, the range was 9.8 mg/ 100 g FW (CO11266-1W/Y, OR) to 32.5 mg/ 100 g FW (Ranger Russet, CO).

Table 4.2: Summary for Year 2 study results, six selected potato genotypes grown in three locations[a].

Genotype	**Location**	**Initial AsA Content[bc]**	**Final AsA Content[bc]**	**Mean**	**Slope**	**AsA Loss (%)**
CO10064-1W/Y	CO	15.4	11.1	13.1	-0.0826	28.0
CO10064-1W/Y	OR	19.3	12.3	16.0	-0.1146	36.1
CO10064-1W/Y	TX	17.7	11.9	14.3	-0.1058	32.7
CO11266-1W/Y	CO	24.1	18.3	21.0	-0.1257	24.0
CO11266-1W/Y	OR	17.5	9.8	13.4	-0.1493	43.7
CO11266-1W/Y	TX	22.1	11.1	16.2	-0.2152	49.9
Ranger Russet	CO	34.0	32.5	29.7	-0.0508	4.5
Ranger Russet	OR	26.5	18.3	21.7	-0.1461	30.8
Ranger Russet	TX	30.9	15.8	21.0	-0.2936	48.9
Rio Grande Russet	CO	35.7	23.7	28.5	-0.1875	33.6
Rio Grande Russet	OR	30.5	18.3	25.0	-0.2226	40.2
Rio Grande Russet	TX	40.0	27.8	32.5	-0.2262	30.5
Sangre-S10	CO	18.7	13.7	16.5	-0.0760	26.5
Sangre-S10	OR	20.6	14.0	17.3	-0.1191	32.0
Sangre-S10	TX	27.4	15.0	21.0	-0.2222	45.1
Yukon Gold	CO	34.4	20.1	25.2	-0.2675	41.7
Yukon Gold	OR	31.3	15.0	22.1	-0.2810	52.0
Yukon Gold	TX	28.1	16.7	23.3	-0.2334	40.6
Mean						35.6

[a] Locations: Colorado, Oregon, Texas.
[b] The AsA content for each entry in this table was an average of 5 biological replicates.
[c] mg/100 g FW, fresh weight.

The average AsA loss (%) across all genotypes and locations was found to be 35.6%. Among the genotypes, Yukon Gold from Oregon exhibited the highest AsA loss at 52.0%, while Ranger Russet from San Luis Valley demonstrated the lowest loss at 4.5%. Ranger Russet potatoes grown in Colorado showed both higher initial AsA content and unusually low AsA loss compared to the same genotype cultivated in two other locations. The reasons for these differences remain unknown, and no prior research papers with similar retention patterns were found. Overall, the initial AsA content observed in this year's study demonstrated noteworthy consistency with the data from the Western Regional Trial spanning 1997-2021. This alignment will be further discussed in section 4.5 and is presented in Table 4.10.

The combined ANOVA for all the genotypes and locations (Table 4.3) revealed evidence of variations in AsA content among the genotypes over the storage period. Additionally, the interaction between genotype and location (environment) was observed, indicating variations in AsA content among the genotypes across different locations. This suggests that environmental conditions and management practices specific to each location influenced the effect of the genotype. The three-way interaction effect involving Genotype, Time, and Location was not found to be statistically significant with respect to its influence on AsA content.

Table 4.3: Combined ANOVA summary table, six selected potato entries grown in 2022 in three locations[a].

Variable	**Sum Sq**	**Df**	**F value**	**Pr(>F)[b]**
(Intercept)	90295	1	6905.1159	< 2.2e-16 ***
Genotype	4840	5	74.0203	< 2.2e-16 ***
Time	5388	1	412.0702	< 2.2e-16 ***
Location	252	2	9.6426	8.074e-05 ***
Genotype:Time	509	5	7.7920	5.062e-07 ***
Genotype:Location	664	10	5.0789	5.337e-07 ***
Time:Location	233	2	8.8947	0.0001652 ***
Genotype:Time:Location	230	10	1.7598	0.0659782

[a] Locations: Colorado, Oregon, Texas.
[b] Significance level: *p<0.05, **p<0.01, ***p<0.001.

The differences in AsA degradation rate discussed above are also illustrated in Figure 4.1.

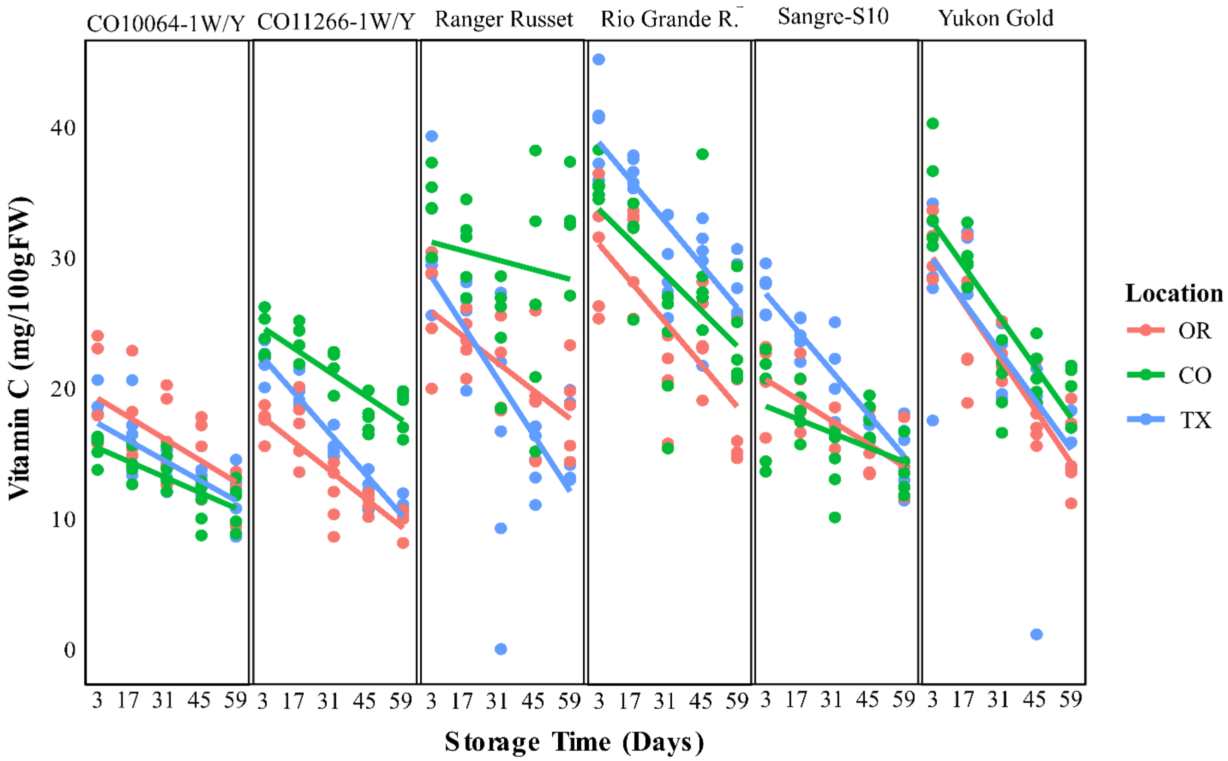

Figure 4.1: Scatterplot comparing AsA degradation for six selected potato entries grown in 2022 in three locations at each storage time point.
[a] CO = Colorado, OR = Oregon, TX= Texas.
[b] Rio Grande R., Rio Grande Russet
FW, fresh weight.

Two main trends were observed in the data. The first trend was characterized by certain genotypes (Ranger Russet, Sangre-S10, CO11266-1W/Y), which displayed non-parallel slopes, sometimes even crossing each other (as seen in Ranger Russet). This suggests that AsA degradation in these genotypes is influenced by the growing environment (time:location or time:environment interaction). These observations were supported by separate ANOVA models individually fitted for each genotype across the three locations and the results of pair-wise comparisons.

The second trend was observed in the other three genotypes (CO10064-1W/Y, Rio Grande Russet, and Yukon Gold) exhibiting similar and almost parallel slopes. The similarity in slope indicates the absence of time:location interaction, and it supports that AsA decreases roughly uniformly over time. Additionally, Rio Grande Russet generally exhibits higher AsA levels throughout the storage period. These observations were supported by separate ANOVA models fitted individually for each genotype (Tables 4.4. – 4.9.) and results of pair-wise comparison.

Table 4.4: ANOVA summary for CO10064-1W/Y.

Genotype	**Variable**	**Sum Sq**	**DF**	**F value**	**Pr(>F)**[a]
CO10064-1W/Y	Intercept	6741.2	1	1338.1062	< 2.2e-16 ***
	Time	300.1	1	59.5643	6.559e-11 ***
	Location	55.0	2	5.4625	0.006277 **
	Time:Location	5.4	2	0.5316	0.590052
	Residuals	347.6	69		

[a]Significance level: *p<0.05, **p<0.01, ***p<0.001.

Based on the ANOVA tests, we did not find evidence of a location by time interaction (F = 0.5316, p = 0.590052) for CO10064-1W/Y. Through pairwise comparison, we didn't find a difference between any slopes comparing the three locations. Texas exhibits a slope of -0.1058, Oregon of -0.1146, and Colorado of -0.0826. However, we found evidence of a difference between intercepts when comparing Oregon and Colorado (estimated difference = 3.9, p = 0.0043).

Table 4.5: ANOVA summary for CO11266-1W/Y.

Genotype	**Variable**	**Sum Sq**	**DF**	**F value**	**Pr(>F)**[a]
CO11266-1W/Y	Intercept	2366.55	1	838.3396	< 2.2e-16 ***
	Time	218.57	1	77.4283	6.922e-13 ***
	Location	177.92	2	31.5142	1.894e-10 ***
	Time:Location	42.21	2	7.4761	0.001151 **
	Residuals	194.78	69		

[a]Significance level: *p<0.05, **p<0.01, ***p<0.001.

Based on the ANOVA tests, we found evidence of a location by time interaction (F = 7.4761, p = 0.001151) for CO11266-1W/Y. For Oregon, the estimated slope corresponding to AsA degradation was -0.1490; for Colorado it was -0.1260; for Texas it was -0.2150. Through pairwise comparison, we found evidence of a difference between slopes comparing Oregon and Texas (estimated difference = 0.0659, p = 0.0207). We also found a difference between slopes comparing Colorado and Texas (estimated difference = 0.0896, p = 0.0011). We found evidence of a difference between intercepts when comparing two pairs of locations: Oregon and Colorado (estimated difference = -6.83, p = <.0001), Oregon and Texas (estimated difference = -4.76, p = <.0001).

Table 4.6: ANOVA summary for Ranger Russet.

Genotype	**Variable**	**Sum Sq**	**DF**	**F value**	**Pr(>F)[a]**
Ranger Russet	Intercept	4998.7	1	173.8027	< 2.2e-16 ***
	Time	209.2	1	7.2746	1.747e-06 ***
	Location	94.1	2	1.6352	0.202369
	Time:Location	293.5	2	5.103	0.008573 **
	Residuals	1984.5	69	-	-

[a]Significance level: *p<0.05, **p<0.01, ***p<0.001.

Based on the ANOVA tests, we found evidence of a location by time interaction (F = 5.103, p = 0.008573) for Ranger Russet. For Oregon, the estimated slope corresponding to AsA degradation was -0.1461; for Colorado it was -0.0507; for Texas it was -0.2936. Through pairwise comparison, we found evidence of a difference between slopes comparing Colorado and Texas (estimated difference = 0.2429, p = 0.0063). However, we did not find evidence of a difference between intercepts for any locations.

Table 4.7: ANOVA summary for Rio Grande Russet.

Genotype	**Variable**	**Sum Sq**	**DF**	**F value**	**Pr(>F)[a]**
Rio Grande Russet	Intercept	26872.1	1	1427.8762	< 2.2e-16 ***
	Time	1322.4	1	70.2651	3.994e-12 ***
	Location	229.1	2	6.0856	0.003681 **
	Time:Location	8.9	2	0.2374	0.789355
	Residuals	1298.6	69	-	-

[a]Significance level: *p<0.05, **p<0.01, ***p<0.001.

Based on the ANOVA tests, we did not find evidence of a location by time interaction (F = 0.2374, p = 0.789355) for Rio Grande Russet. For Oregon, the estimated slope corresponding to AsA degradation was -0.2230; for Colorado it was -0.1880; for Texas it was -0.2260. Through pairwise comparison, we didn't find a difference between any slopes comparing the three locations. However, we found evidence of a difference between intercepts when comparing Oregon and Texas (estimated difference = -7.80, p = 0.0030).

Table 4.8: ANOVA summary for Sangre-S10.

Genotype	**Variable**	**Sum Sq**	**DF**	**F value**	**Pr(>F)[a]**
Sangre-S10	Intercept	11056.8	1	1928.994	< 2.2e-16 ***
	Time	569.1	1	99.292	5.401e-15 ***
	Location	325.4	2	28.382	1.013e-09 ***
	Time:Location	110.5	2	9.640	0.0002033 ***
	Residuals	395.5	69	-	-

[a] Significance level: *p<0.05, **p<0.01, ***p<0.001.

Based on the ANOVA tests, we found evidence of a location by time interaction (F = 9.640, p = 0.0002033) for Sangre-S10. For Oregon, the estimated slope corresponding to AsA degradation was -0.1190; for Colorado it was -0.0760; for Texas it was -0.2220. Through pairwise comparison, we found evidence of a difference between slopes comparing Oregon and Texas (estimated difference = 0.1030, p = 0.0100). We also found a difference between slopes comparing Colorado and Texas (estimated difference = 0.1461, p = 0.0002). We found evidence of a difference between intercepts when comparing two pairs of locations: Oregon and Texas (estimated difference = -6.95, p = <0.0001), and Colorado and Texas (estimated difference = -9.06, p = <0.0001).

Table 4.9: ANOVA summary for Yukon Gold.

Genotype	Variable	Sum Sq	DF	F value	Pr(>F)[a]
Yukon Gold	Intercept	21770.0	1	1269.3750	<2e-16 ***
	Time	2135.3	1	124.5035	<2e-16 ***
	Location	37.0	2	1.0776	0.3461
	Time:Location	2.2	2	0.0642	0.9379
	Residuals	1183.4	69	-	-

[a]Significance level: $*p<0.05$, $**p<0.01$, $***p<0.001$.

Based on the ANOVA tests, we did not find evidence of a location by time interaction ($F = 0.0642$, $p = 0.9379$) for Yukon Gold. For Oregon, the estimated slope corresponding to AsA degradation was -0.2810; for Colorado it was -0.2670; for Texas it was -0.2600. Through pairwise comparison, we didn't find a difference between any slopes comparing all three locations. We also did not find evidence of a difference between intercepts when comparing all locations.

4.5. Discussion

The impact of the environment and growing practices on AsA levels and retention during storage was evident in our study, which is consistent with other research papers (Burgos et al., 2009; Thomas et al., 2021). Among the genotypes examined, three showed evidence of variation between AsA retention and growing location (time:environment interaction), as indicated by varying slopes, which is supported by previous publications (Hamouz et al., 2007; Valcarcel et al., 2015a; Valcarcel et al., 2015b).

Four genotypes demonstrated variation in initial AsA content over three different locations, representing a genotype:environment (GxE) interaction. Future extensive research with a larger number of genotypes and diverse locations could shed light on genotypes that display better consistency or adaptability to various environmental conditions, ensuring better nutritional composition, including AsA. Additionally, such studies may reveal specific genotypes that perform optimally in particular locations of interest. Moreover, the data for six genotypes from

SLVRC, CO this year aligned well with the Western Regional Trials data from 1998-2021 (USDA, 1998-2021), enhancing the overall comparability and consistency of our findings.

Furthermore, exploring longer storage times could offer valuable insights into the AsA retention patterns of genotypes that initially exhibited strong retention. By studying how genotypes behave under extended storage, we can better assess their suitability for practical applications and potentially identify those with enhanced AsA retention capabilities.

It's important to note that different potato varieties have varying dormancy lengths, followed by a sprouting stage that is accompanied by changes in metabolism. Investigating and accounting for this process is crucial for gaining a better understanding of AsA retention and the factors that influence it.

Rio Grande Russet generally exhibited a higher initial AsA content and an unusually low AsA loss during the storage period compared to that grown in Oregon and Texas. The reasons for these differences remain unknown, and no prior research papers with similar retention patterns were found. This requires further investigation.

4.6. Comparison of Year 1 and Year 2 Results with Western Regional Trial Reports

Preceding the study discussed above, an analysis of the Western Regional Trial Reports covering the period from 1997 to 2021 was conducted to identify relevant genotypes for the Year 1 study. Consequently, 34 genotypes were selected for Year 1, and among them, 6 genotypes were retained for the Year 2 study. This allowed us to compare our findings from 2 years of research with the Western Regional Trials to assess the consistency. The results of this comparison are presented in Table 4.10.

Table 4.10: Overall AsA content comparison of six potato entries, Western Regional Trial (1997-2021); SLVRC[a], CO, 2021 and 2022.

AsA content (mg/100 g FW[b])			
Genotype	Western Regional Trial	Year 2021	Year 2022
Yukon Gold	38.6	22.1	34.4
Rio Grande Russet	35.6	28.0	36.4
Ranger Russet	32.0	25.0	34.0
CO10064-1W/Y	16.9	10.1	15.4
Sangre-S10	16.5	20.4	18.7
CO11266-1W/Y	15.8	15.9	24.0

[a]SLVRC = San Luis Valley Research Center.

[b]FW, fresh weight.

The results obtained in 2022 show are very comparable in AsA level data to the Western Regional Trial Reports. Generally, data from 2021 showed compared to the results from 2021. Further investigation is required to ascertain the reasons behind the observed differences in comparability. The hail observed in 2021 may have been one of the factors involved.

4.7. Conclusions

In conclusion, the findings of this study highlight the importance of considering both genotype and storage time in retention of AsA content in potato tubers. The degradation of AsA during storage varied among the studied genotypes, emphasizing the need to select genotypes with better retention. Although, environment (location) significantly influences AsA degradation for three out of six genotypes, it is still crucial to adopt appropriate storage time and conditions to minimize AsA loss. Further research and practical interventions can focus on identifying genotypes with better AsA retention characteristics and optimizing storage conditions to preserve the nutritional value of potato tubers for consumers. Understanding the mechanisms of AsA accumulation and retention in potatoes holds the potential to enhance their nutritional composition.

The observation that AsA content is influenced by genotype provides a strong basis for considering the use of breeding strategies to increase AsA levels by selecting genotypes with inherently higher content.

Recognizing that genotype-environment interactions control AsA levels implies that we can tailor breeding efforts to specific geographical regions. For example, by breeding genotypes that are well-suited to the unique environmental conditions of Colorado, we could potentially increase AsA levels in potatoes grown within this region, thereby addressing local nutritional needs more effectively.

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LIST OF ABBREVIATIONS

AsA	ascorbic acid
CDC	Centers for Disease Control and Prevention
CSU	Colorado State University
DHA	dehydroascorbic acid
DKG	diketogulonate
FAO	Food and Agriculture Organization
FDA	Food and Drug Administration
FW	fresh weight
g	grams
HPLC	HPLC high-performance liquid chromatography
mg	milligram
mL	milliliter
RDA	recommended daily allowance
SD	standard deviation
SLVRC	San Luis Valley Research Center
USDA	United States Department of Agriculture-Agricultural Research Service
µg	micrograms
µL	microliter
µm	micrometer