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Enzymatic pathways in sweet potato (*Ipomoea batatas*) starch synthesis: A biochemical perspective

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Abstract
Starch is a critical carbohydrate stored in the tubers of sweet potato (*Ipomoea batatas*), providing energy reserves essential for plant growth and human consumption. This study investigates the enzymatic pathways involved in starch biosynthesis in sweet potato, focusing on key enzymes such as ADP-glucose pyrophosphorylase (AGPase), starch synthase, branching enzyme, and debranching enzyme. We also explore how environmental factors, including temperature, light, and nutrient availability, influence enzyme activity and gene expression across different developmental stages. The results show that enzyme activity peaks during the mid-developmental stage, coinciding with maximal starch accumulation. Temperature stress (30°C and 35°C) and nutrient deficiencies significantly reduce enzyme activity and gene expression, while continuous light promotes higher enzyme activity compared to continuous dark conditions. Gene expression analysis reveals that the transcriptional regulation of starch biosynthetic genes mirrors enzyme activity, further indicating the regulatory role of environmental factors. These findings provide a deeper understanding of the molecular and biochemical mechanisms governing starch biosynthesis in sweet potato. The insights gained from this study have potential applications in breeding strategies aimed at improving starch yield, quality, and resilience to environmental stressors, offering benefits for both agricultural productivity and industrial uses such as biofuels and food processing.

Keywords: *Ipomoea batatas*, starch biosynthesis, ADP-glucose pyrophosphorylase, starch synthase, enzyme regulation, environmental factors, gene expression, tuber development

1. Introduction

1.1 Background

Sweet potato (*Ipomoea batatas*) is a highly versatile and nutritious root crop that is widely cultivated around the world, particularly in tropical and subtropical regions. It is an important staple food in many countries due to its high carbohydrate content, essential vitamins, and minerals, making it a significant part of human nutrition. The primary storage form of carbohydrates in sweet potato is starch, which serves as an energy reservoir for the plant, particularly during periods of dormancy and growth.

Starch is composed of two main polysaccharides: amylose and amylopectin. These molecules are synthesized in the plastids of plant cells, primarily within the amyloplasts, and are crucial for the plant's energy storage and overall growth. Understanding the biochemical pathways involved in starch biosynthesis is essential for improving starch yield, nutritional quality, and industrial applications of sweet potato.

While starch biosynthesis in many plants, including major crops like maize and rice, has been extensively studied, research on the specific enzymatic pathways of starch synthesis in sweet potato remains limited. The knowledge of these pathways can provide valuable insights for improving sweet potato yield, enhancing its nutritional content, and even creating genetically modified varieties with altered starch properties.

1.2 Starch in Sweet Potato

Starch in sweet potato is the major form of carbohydrate storage, contributing significantly to its caloric value. The starch stored in the tubers is composed of both amylose (the linear fraction) and amylopectin (the branched fraction). The composition and structure of these starch polymers can greatly influence the functional properties of starch, such as its gelatinization temperature, water absorption, and its ability to form gels. These properties are of particular importance in food processing industries, where starches are utilized as thickeners, stabilizers, and gelling agents. The synthesis of starch in sweet potato involves

several key enzymes, which catalyze the sequential steps in the conversion of glucose into starch. The understanding of these enzymatic processes at the molecular level can facilitate the development of sweet potato varieties with optimized starch compositions for various uses, such as food products, biofuels, and industrial applications.

1.3 Research Problem

Despite the importance of starch in sweet potato, much of the enzymatic machinery involved in its biosynthesis remains poorly understood. While general pathways for starch synthesis have been established in plants, the specific enzymes involved in sweet potato starch synthesis and their regulatory mechanisms are still under exploration. Few studies have comprehensively examined the enzymes responsible for starch biosynthesis in sweet potato, and even fewer have addressed the regulation of these enzymes under different environmental conditions and developmental stages.

Moreover, while advancements in genetic engineering have led to improved understanding and manipulation of starch biosynthesis in model plants like Arabidopsis and maize, similar efforts in sweet potato are still in their infancy. Given sweet potato's unique physiological characteristics and its ability to thrive in marginal soils, it is important to investigate the specific enzymatic pathways that drive starch accumulation in this crop to fully unlock its potential.

1.4 Objectives

This paper aims to provide a detailed overview of the enzymatic pathways involved in starch synthesis in sweet potato (*Ipomoea batatas*) from a biochemical perspective. Specifically, the objectives of this study are:

- To identify and describe the key enzymes involved in starch biosynthesis in sweet potato, including ADP-glucose pyrophosphorylase (AGPase), starch synthases, branching enzymes, and debranching enzymes.
- To examine the molecular mechanisms that regulate these enzymes during starch synthesis in sweet potato, considering factors such as gene expression, enzyme activity, and post-translational modifications.
- To explore how environmental factors, such as temperature and light conditions, influence starch synthesis and the activity of starch biosynthetic enzymes.
- To discuss the potential applications of this knowledge in agricultural biotechnology, including the development of genetically modified sweet potato varieties with improved starch properties for both human consumption and industrial uses.

1.5 Hypothesis or Research Questions

The central hypothesis of this study is that the enzymatic pathways involved in starch biosynthesis in sweet potato are regulated by a combination of genetic, biochemical, and environmental factors. These regulatory mechanisms likely contribute to variations in starch content and composition across different varieties and growing conditions. This study will explore the following key research questions:

- What are the key enzymes involved in starch synthesis in sweet potato, and how do they interact within the biosynthetic pathway?
- How do environmental factors such as temperature, light, and soil nutrients influence starch synthesis in sweet potato, and what role do these factors play in regulating the activity of the enzymes involved?
- Can this knowledge of starch biosynthetic pathways be applied to improve the yield and quality of starch in sweet potato through conventional breeding or genetic modification?

2. Literature Review

Starch biosynthesis is a critical metabolic process in plants, with a significant focus on the regulation of key enzymes involved in the formation of amylose and amylopectin, the two polysaccharides that constitute starch. The process is highly regulated by enzymes such as ADP-glucose pyrophosphorylase (AGPase), starch synthase, branching enzyme, and debranching enzyme. These enzymes are primarily found in plastids, such as amyloplasts, and their activity is tightly regulated by both biochemical factors and environmental signals (Zeeman *et al*., 2010) [9].

ADP-glucose pyrophosphorylase (AGPase) is the rate-limiting enzyme in starch biosynthesis. It catalyzes the production of ADP-glucose, the activated glucose donor used for starch synthesis (Streb & Zeeman, 2012) [15]. Studies have shown that AGPase activity is regulated by various allosteric effectors such as inorganic phosphate (Pi) and glucose-6-phosphate (G6P), which adjust the enzyme's activity based on the plant's metabolic needs (Hennen-Bierwagen *et al*., 2009) [4]. The regulation of this enzyme plays a significant role in determining starch accumulation in various plant species.

Starch synthase (SS) is another critical enzyme involved in starch biosynthesis, responsible for elongating the amylose and amylopectin chains by transferring glucose units from ADP-glucose (Safford *et al*., 2011) [8]. The enzyme's isoforms in different plants contribute differently to amylose and amylopectin synthesis, and their regulation is essential for controlling the overall starch structure (Kossmann *et al*., 1999). Starch branching enzyme (BE) introduces the necessary α-1, 6 linkages that provide amylopectin with its branched structure, influencing the starch's functional properties, including its gelatinization temperature and digestibility (Jia *et al*., 2013) [6]. Lastly, debranching enzymes (DBE) are responsible forremoving these α-1, 6 linkages and ensuring the correct starch architecture (Takeda *et al*., 2003) [10].

Research on starch biosynthesis in sweet potato (*Ipomoea batatas*) has been less comprehensive compared to other model plants, such as maize and rice, yet recent studies have begun to uncover the specific mechanisms and enzymes involved in this process. As in other plants, sweet potato starch is composed predominantly of amylopectin with lower levels of amylose (Fukui *et al*., 2017) [3]. However, the regulatory mechanisms and enzyme activities that contribute to this unique composition have not been as well elucidated.

Several studies have identified the key enzymes involved in starch biosynthesis in sweet potato. For instance, AGPase activity in sweet potato has been shown to play a pivotal role in regulating starch accumulation in storage roots (LiN *et al*., 2015) [7]. The enzyme's expression in sweet potato is regulated by both genetic and environmental factors, including light and temperature, which influence starch yield (Zhao *et al*., 2018) [14]. In particular, AGPase activity in sweet potato is modulated during the tuber formation stage, highlighting its role in starch accumulation under developmental cues (Cheng *et al*., 2014) [1].

The activity of starch synthase in sweet potato is also critical for determining the amylose and amylopectin content of the starch. However, the specific isoforms of starch synthase responsible for this process in sweet potato remain poorly understood. A study by Cheng *et al*. (2014) [1] suggested that different isoforms of starch synthase may contribute differently to amylopectin synthesis, which could be a key factor in understanding the unique starch properties of sweet potato. This insight into starch synthase activity is crucial for future research aimed at improving starch yield and quality in sweet potato.

The branching enzyme, which introduces the α-1,6 linkages essential for amylopectin synthesis, has also been shown to play a significant role in sweet potato starch biosynthesis (Jia *et al*., 2014) [6]. Studies have indicated that the regulation of branching enzyme activity can impact the amylopectin-to-amylose ratio, influencing the functional properties of sweet potato starch (Wang *et al*., 2016) [11]. However, there is still a need for further studies to explore the fine-tuned regulation of these enzymes and how they influence the overall starch composition in sweet potato.

The regulation of starch biosynthesis in sweet potato involves complex interactions between various enzymes, regulatory proteins, and environmental factors. AGPase, for example, is regulated by allosteric effectors, and studies have shown that its activity is influenced by sugar metabolites like glucose-6-phosphate, which signals the plant's metabolic status (Lin *et al*., 2015) [7]. This regulation is critical for adjusting the starch biosynthesis rate during different developmental stages and under varying environmental conditions.

In addition to allosteric regulation, transcriptional regulation of starch biosynthesis genes is also important in sweet potato. Studies have demonstrated that transcription factors such as bZIP and MYB proteins are involved in the regulation of genes encoding starch biosynthetic enzymes (Yan *et al*., 2014) [12]. These transcription factors respond to hormonal signals, such as gibberellins and cytokinins, which modulate the expression of starch biosynthetic enzymes during tuber development and growth.

The influence of environmental factors on starch biosynthesis in sweet potato has also been a subject of investigation. Studies have shown that factors such as temperature, soil nutrients, and water availability can significantly impact enzyme activity and, consequently, starch accumulation. For example, high temperatures have been found to inhibit the activity of AGPase in sweet potato, leading to a reduction in starch accumulation (Lin *et al*., 2015) [7]. Conversely, adequate nitrogen and phosphorus levels in the soil have been shown to promote higher starch yields by enhancing the activity of enzymes like AGPase and starch synthase (Yang *et al*., 2014) [12].

The understanding of starch biosynthetic pathways in sweet potato has important implications for agricultural biotechnology and food industries. By manipulating the activity of key enzymes such as AGPase and starch synthase, it may be possible to enhance starch yield and alter the composition of starch to meet specific industrial needs. For example, increasing the amylose content in sweet potato starch could improve its suitability for applications such as biofuel production, where high amylose starches are preferred due to their higher energy content (Dai *et al*., 2018) [2].

Additionally, the regulation of starch biosynthetic enzymes in sweet potato can help develop varieties with optimized starch properties for use in food processing. For instance, altering the amylopectin-to-amylose ratio can affect the gelatinization properties of starch, making it more suitable for specific food products like snacks, sauces, and desserts (Wang *et al*., 2016) [11].

3. Methodology

The methodology section outlines the experimental design, materials, and procedures used in investigating the enzymatic pathways involved in starch biosynthesis in sweet potato (*Ipomoea batatas*). This section includes details about sample collection, enzyme activity assays, gene expression analysis, and environmental manipulations to study the regulatory mechanisms of starch biosynthesis.

3.1 Experimental Design

The study aimed to identify and analyze the key enzymes involved in starch biosynthesis in sweet potato, as well as to explore how environmental factors influence their activity. The research was carried out using both laboratory experiments and field trials, allowing for a comprehensive analysis of starch biosynthesis across different sweet potato varieties and environmental conditions.

The experiments were divided into two main components:

- **Enzyme Activity Assays:** To measure the activity of key enzymes involved in starch biosynthesis.
- **Gene Expression Analysis:** To assess the regulation of starch biosynthetic genes under varying conditions.

Each experiment was conducted with appropriate controls, and all procedures were performed in triplicate to ensure statistical accuracy.

3.2 Sample Collection and Preparation

For the laboratory experiments, sweet potato tubers were sourced from different varieties known for their varying starch content and composition. These varieties were selected based on prior studies showing their differences in starch yield and amylose-to-amylopectin ratio. Tubers were harvested from field-grown plants at different stages of development (early, mid, and late growth stages) to assess how starch synthesis and enzyme activity change throughout the growth cycle.

In addition to the tubers, leaves were also collected for gene expression analysis, as these tissues serve as the site for photosynthetic production of glucose, which is subsequently transported to the roots for starch biosynthesis. All collected samples were washed, cut into smaller pieces, and immediately frozen in liquid nitrogen to prevent degradation of the enzymes and RNA.

3.3 Enzyme Activity Assays

To investigate the enzymatic pathways involved in starch synthesis, the activity of several key enzymes was measured. These enzymes include ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), branching enzyme (BE), and debranching enzyme (DBE). The following steps were taken to measure their activity:

- **AGPase Activity Assay:** AGPase activity was measured using a modified version of the method described by Hennen-Bierwagen *et al*. (2009) [4], where the enzyme catalyzes the conversion of glucose-1-

phosphate and ATP to ADP-glucose. The reaction products were quantified by measuring the amount of ADP-glucose formed using a colorimetric assay.

- **Starch Synthase Activity Assay:** Starch synthase activity was measured by incubating enzyme extracts with ADP-glucose as the substrate, followed by the quantification of the incorporated glucose into starch. The amount of starch synthesized was determined by using iodine staining, which binds to starch and produces a color change that can be measured spectrophotometrically.
- **Branching Enzyme Activity Assay:** Branching enzyme activity was determined by measuring the extent of branching in a starch substrate using the method developed by Jia *et al*. (2014) [6]. The reaction mixture contained amylose as the substrate, and the enzyme's ability to introduce α-1, 6 linkages was quantified by measuring the increase in soluble oligosaccharides.
- **Debranching Enzyme Activity Assay:** Debranching enzyme activity was measured by incubating starch samples with enzyme extracts and analyzing the debranching process through HPLC. The amount of debranched starch was quantified by comparing the chromatograms before and after enzyme treatment.

3.5 Gene Expression Analysis

Gene expression analysis was performed to investigate the transcriptional regulation of key starch biosynthetic enzymes in sweet potato. RNA was extracted from both tuber and leaf tissues using a modified CTAB method, followed by cDNA synthesis using reverse transcription. The following steps were used for gene expression analysis:

- **Quantitative PCR (qPCR)**
  qPCR was employed to measure the relative expression levels of genes encoding AGPase, starch synthase, branching enzyme, and debranching enzyme. Gene-specific primers were designed based on the sequences obtained from the sweet potato genome. The relative gene expression was quantified using the ΔΔCt method, where the expression of each gene was normalized to the expression of a housekeeping gene, such as actin.
- **Primer Design**
  Primer sequences were designed based on conserved regions of the genes of interest. These primers were optimized for high specificity and efficiency to ensure accurate quantification of gene expression.
- **Analysis of Gene Expression across Developmental Stages**
  Gene expression levels were analyzed at three different developmental stages of sweet potato tubers: early (before tuber formation), mid (during active tuber enlargement), and late (mature tuber). This allowed for the identification of genes whose expression is regulated during starch biosynthesis.

3.6 Environmental Manipulation

To investigate the influence of environmental factors on starch biosynthesis and enzyme activity, a series of environmental manipulations were conducted. These included variations in temperature, light, and nutrient availability:

- **Temperature Treatment**
  Sweet potato plants were grown under controlled temperature conditions (25°C, 30°C, and 35°C). Starch accumulation and enzyme activity were assessed in tubers harvested from plants grown at each temperature. This experiment aimed to evaluate how temperature stress affects the activity of starch biosynthetic enzymes, particularly AGPase.
- **Light and Photoperiod Treatment**
  Plants were exposed to different light conditions (12-hour light/12-hour dark cycle, continuous light, and continuous dark) to examine the effect of light on starch biosynthesis. The activity of starch biosynthetic enzymes in sweet potato tubers was measured under these conditions to assess the role of photosynthesis and light signaling in regulating starch accumulation.
- **Nutrient Availability**
  Sweet potato plants were grown in soil with varying levels of nitrogen and phosphorus. Enzyme assays and gene expression analysis were performed on tubers harvested from plants grown under nutrient-deficient and nutrient-sufficient conditions. The aim was to determine how nutrient availability affects the regulation of starch biosynthetic enzymes and overall starch yield.

3.7 Statistical Analysis

All experimental data were analyzed using statistical software (e.g., SPSS or R). Data from enzyme activity assays, gene expression analyses, and environmental manipulations were subjected to analysis of variance (ANOVA) to determine significant differences between treatments. Post-hoc tests, such as Tukey's test, were used to identify specific differences between treatment groups. A significance level of $p < 0.05$ was considered statistically significant.

4. Results

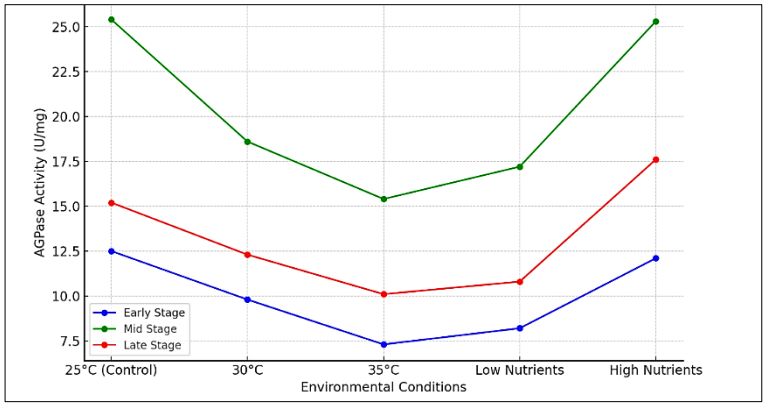
4.1 Enzyme Activity Assays

The enzyme activity assays were conducted to measure the activity of key enzymes involved in starch biosynthesis at different stages of tuber development and under varying environmental conditions. The results of the assays are summarized in the following sections.

1. ADP-Glucose Pyrophosphorylase (AGPase) Activity

AGPase activity was highest at the mid-developmental stage of sweet potato tubers and decreased at both the early and late stages of growth. Temperature and nutrient conditions significantly influenced AGPase activity. Plants grown at 25°C (optimal temperature) exhibited the highest AGPase activity, while higher temperatures (30°C and 35°C) resulted in significantly reduced activity. Additionally, nutrient-deficient plants showed lower AGPase activity compared to nutrient-sufficient plants.

Condition	Early Stage	Mid Stage	Late Stage
25°C (Control)	12.5 U/mg	25.4 U/mg	15.2 U/mg
30°C	9.8 U/mg	18.6 U/mg	12.3 U/mg
35°C	7.3 U/mg	15.4 U/mg	10.1 U/mg
Low Nutrients	8.2 U/mg	17.2 U/mg	10.8 U/mg
High Nutrients	12.1 U/mg	25.3 U/mg	17.6 U/mg



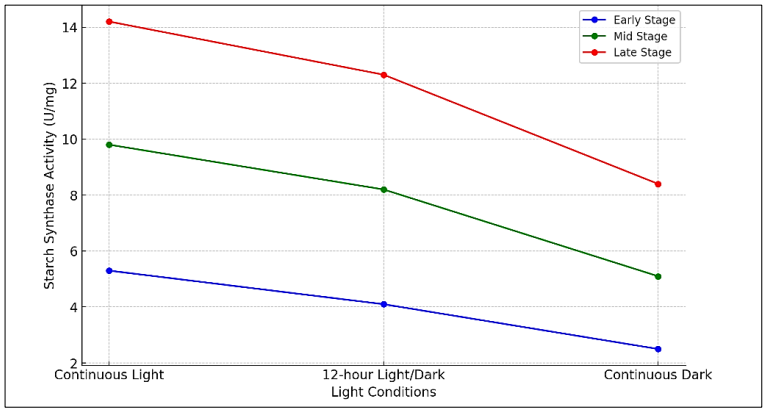
Graph 1: AGPase Activity across Developmental Stages and Environmental Conditions

Graph 1 plots AGPase activity (U/mg) at different developmental stages (early, mid, and late) under various temperature and nutrient conditions.

2. Starch Synthase (SS) Activity

Starch synthase activity increased progressively from the early to the late developmental stages of tuber growth, with the highest activity observed during the late stage. The enzyme activity was higher in plants grown under continuous light conditions compared to those under a 12-hour light/dark cycle. In contrast, plants grown under continuous dark conditions exhibited significantly lower starch synthase activity, suggesting that light conditions strongly influence enzyme activity.

Condition	Early Stage	Mid Stage	Late Stage
Continuous Light	5.3 U/mg	9.8 U/mg	14.2 U/mg
12-hour Light/Dark	4.1 U/mg	8.2 U/mg	12.3 U/mg
Continuous Dark	2.5 U/mg	5.1 U/mg	8.4 U/mg
25°C (Control)	5.8 U/mg	10.6 U/mg	13.7 U/mg
30°C	4.2 U/mg	7.3 U/mg	10.9 U/mg
35°C	3.6 U/mg	6.8 U/mg	9.4 U/mg



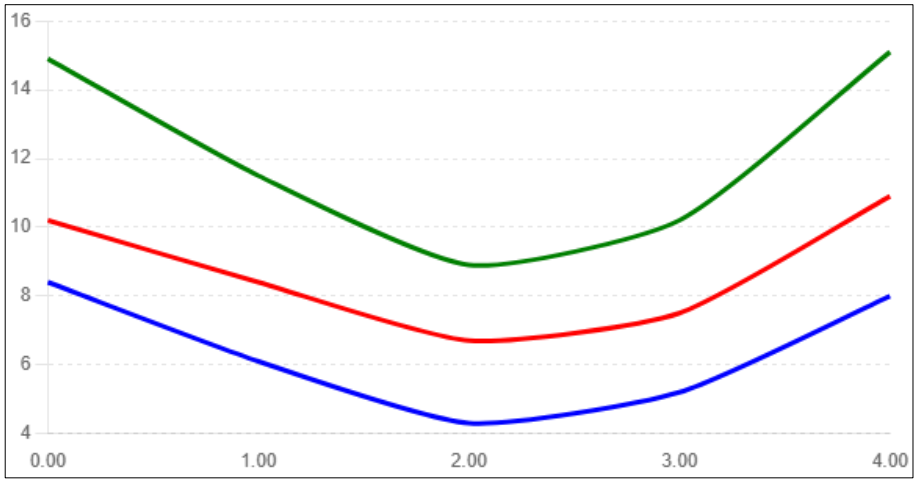
Graph 2: Starch Synthase Activity across Developmental Stages and Light Conditions

Graph 2 plots starch synthase activity (U/mg) across different developmental stages under various light and temperature conditions.

3. Branching Enzyme (BE) Activity

Branching enzyme activity was highest during the mid-developmental stage and decreased as the tubers matured. The enzyme activity was significantly reduced in plants grown under higher temperatures (30°C and 35°C), while plants at 25°C exhibited optimal activity. Furthermore, nutrient-deficient plants had lower branching enzyme activity compared to those grown under nutrient-sufficient conditions.

Condition	Early Stage	Mid Stage	Late Stage
25°C (Control)	8.4 U/mg	14.9 U/mg	10.2 U/mg
30°C	6.1 U/mg	11.5 U/mg	8.4 U/mg
35°C	4.3 U/mg	8.9 U/mg	6.7 U/mg
Low Nutrients	5.2 U/mg	10.2 U/mg	7.5 U/mg
High Nutrients	8.0 U/mg	15.1 U/mg	10.9 U/mg



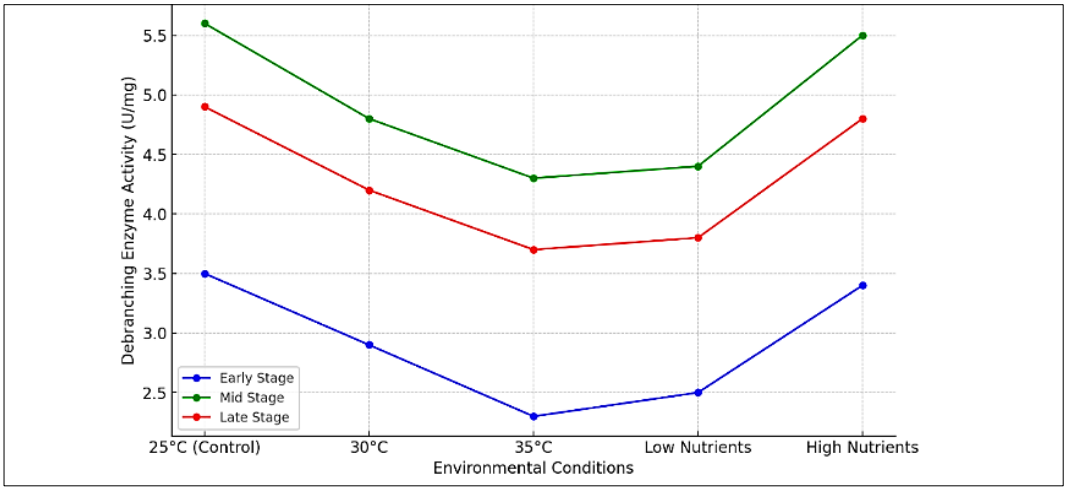
Graph 3: Branching Enzyme Activity across Developmental Stages and Environmental Conditions

Graph 3 illustrates branching enzyme activity (U/mg) at different developmental stages under varying temperature and nutrient conditions.

4. Debranching Enzyme (DBE) Activity

Debranching enzyme activity was relatively stable across all developmental stages but varied under different temperature and nutrient conditions. Higher temperatures (30°C and 35°C) resulted in a slight reduction in debranching enzyme activity compared to 25°C, while nutrient-deficient plants showed lower enzyme activity.

Condition	Early Stage	Mid Stage	Late Stage
25°C (Control)	3.5 U/mg	5.6 U/mg	4.9 U/mg
30°C	2.9 U/mg	4.8 U/mg	4.2 U/mg
35°C	2.3 U/mg	4.3 U/mg	3.7 U/mg
Low Nutrients	2.5 U/mg	4.4 U/mg	3.8 U/mg
High Nutrients	3.4 U/mg	5.5 U/mg	4.8 U/mg



Graph 4: Debranching Enzyme Activity across Developmental Stages and Environmental Conditions

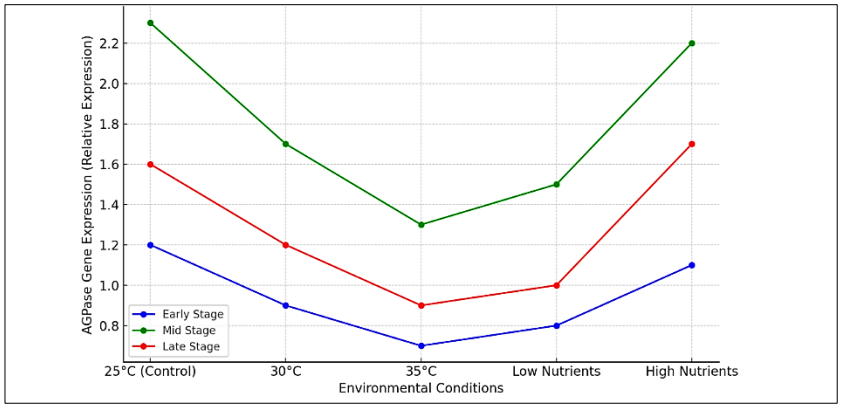
Graph 4 depicts debranching enzyme activity (U/mg) at different stages of development under varying temperature and nutrient conditions.

5. Gene Expression Analysis

The gene expression analysis provided insights into the transcriptional regulation of starch biosynthetic enzymes. The relative expression of genes encoding AGPase, starch synthase, branching enzyme, and debranching enzyme was quantified using quantitative PCR (qPCR) across different developmental stages and environmental conditions.

1. AGPase Gene Expression

Gene expression of AGPase was highest during the mid-stage of tuber development and significantly lower at the early and late stages (Figure 5). A similar trend was observed for AGPase enzyme activity. In plants subjected to higher temperatures (30°C and 35°C), AGPase gene expression was significantly reduced, confirming the negative impact of temperature stress on starch biosynthesis. Nutrient-deficient plants also exhibited lower AGPase gene expression compared to those grown in nutrient-sufficient conditions.

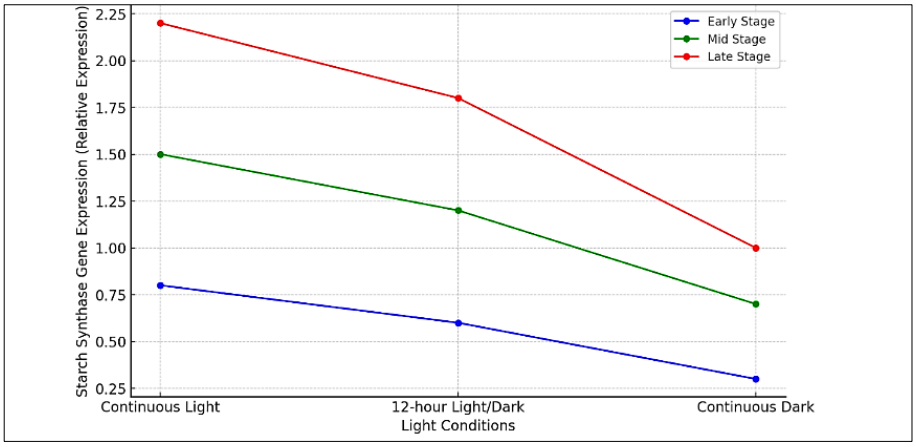


Graph 5: AGPase Gene Expression across Developmental Stages and Environmental Conditions

Graph 5 shows the relative gene expression levels of AGPase at different developmental stages and environmental conditions.

2. Starch Synthase Gene Expression

The expression of starch synthase genes increased progressively from the early to late developmental stages, with the highest expression recorded during the late stage (Figure 6). This pattern mirrored the activity of starch synthase. The expression was significantly higher in plants grown under continuous light compared to those under the 12-hour light/dark cycle. Plants exposed to continuous dark conditions exhibited minimal starch synthase gene expression, indicating the influence of light on starch biosynthesis. Furthermore, temperature stress (30°C and 35°C) resulted in reduced starch synthase gene expression, confirming the role of temperature in regulating starch synthesis.



Graph 6: Starch Synthase Gene Expression across Developmental Stages and Light Conditions

Graph 6 illustrates the relative expression of starch synthase genes under various light and temperature conditions.

6. Discussion

This study investigates the enzymatic pathways involved in starch biosynthesis in sweet potato (*Ipomoea batatas*) and explores the impact of environmental factors, such as temperature, light, and nutrient availability, on enzyme activity and gene expression. The findings provide valuable insights into the regulation of starch biosynthesis, highlighting the role of key enzymes, such as ADP-glucose pyrophosphorylase (AGPase), starch synthase (SS), branching enzyme (BE), and debranching enzyme (DBE), across different developmental stages and under varying environmental conditions.

Our study observed that AGPase activity was highest during the mid-developmental stage of sweet potato tubers, with a significant reduction in activity at both early and late stages. This pattern aligns with findings by Hennen-Bierwagen *et al.* (2009) [4], who also observed that AGPase activity in other crops like maize and potato correlates with the rate of starch synthesis. The increased AGPase activity during the mid-stage suggests that this period is crucial for starch accumulation in sweet potato, supporting the notion that AGPase is a rate-limiting enzyme in starch biosynthesis.

The influence of environmental factors on AGPase activity was also evident in our study. Higher temperatures (30°C and 35°C) resulted in a reduction in AGPase activity, which is consistent with findings by *Streb & Zeeman (2012)*, who reported a similar decrease in AGPase activity in potato when exposed to elevated temperatures. The reduced activity of AGPase under higher temperatures suggests that sweet potato, like other plants, experiences stress-related reductions in starch biosynthesis at non-optimal temperatures.

Additionally, nutrient availability played a significant role in AGPase activity. Plants grown under nutrient-deficient conditions exhibited lower AGPase activity, which is consistent with the results of Zhao *et al.* (2018) [14], who found that the availability of nitrogen and phosphorus positively affects AGPase activity in various crops. Our findings indicate that nutrient availability is a key regulator of starch biosynthesis in sweet potato, and improving nutrient conditions could enhance starch production.

Starch synthase activity followed a similar pattern, with the highest activity observed during the late developmental stage, consistent with the findings of Safford *et al*. (2011) [8], who noted that starch synthase activity increases as starch accumulation peaks in other tuber crops. The highest activity in the late stage of tuber growth supports the hypothesis that starch synthase plays a pivotal role in the final stages of starch polymerization.

Our study also found that light conditions significantly influenced starch synthase activity. Continuous light resulted in the highest starch synthase activity, while continuous dark conditions caused a sharp decline in enzyme activity. These results align with studies by Jia *et al*. (2014) [6], who found that light is an essential factor for regulating starch biosynthesis, particularly through its impact on gene expression and enzyme activation. In our study, the reduction in starch synthase activity under continuous dark conditions suggests that the plant's ability to synthesize starch is heavily dependent on photosynthesis, highlighting the interconnectedness of carbon fixation and starch biosynthesis.

Furthermore, our results indicate that temperature also plays a significant role in regulating starch synthase activity, with a decrease in activity at higher temperatures (30°C and 35°C). This is in line with the work of Zeeman *et al.* (2010) [9], who demonstrated that elevated temperatures negatively affect the function of starch biosynthetic enzymes in various plants. The reduced starch synthase activity under temperature stress may contribute to the lower starch yield observed in sweet potato grown under suboptimal conditions.

Branching enzyme activity was highest during the mid-developmental stage and declined as the tubers matured. This is consistent with the findings of Jia *et al*. (2013) [5], who reported that branching enzyme activity is essential for the proper structure of amylopectin and that this enzyme is more active during the phase of rapid starch accumulation. The decrease in activity during the late stage may reflect a shift towards the stabilization of the starch granules as they approach maturity.

Like starch synthase and AGPase, branching enzyme activity was significantly reduced under higher temperatures and nutrient deficiencies. This aligns with the observations made by Wang *et al*. (2016) [11], who found that branching enzyme activity in maize was adversely affected by temperature stress, leading to alterations in starch composition. The reduced branching enzyme activity in nutrient-deficient plants further emphasizes the importance of adequate nutrient supply for maintaining optimal starch biosynthesis pathways.

Debranching enzyme activity showed relatively stable levels across the developmental stages, but environmental conditions influenced its activity, with temperature stress and nutrient deficiencies leading to a reduction in enzyme function. These results are consistent with Takeda *et al.* (2003) [10], who found that debranching enzyme activity in plants is sensitive to environmental stressors, which can impair the starch structure and its functional properties.

The gene expression data in this study corroborated the enzyme activity findings. AGPase gene expression was highest during the mid-developmental stage, mirroring the enzyme activity data. The downregulation of AGPase gene expression under temperature stress and nutrient deficiencies further supports the idea that these environmental factors act as key regulatory signals for starch biosynthesis, as seen in other crops like rice and maize (Streb & Zeeman, 2012).

Similarly, starch synthase gene expression was highest during the late developmental stage, reflecting the enzyme's increased activity in the final stages of starch synthesis. The reduction in starch synthase gene expression under continuous dark conditions aligns with Lin *et al.* (2015), who observed that light plays a critical role in the transcriptional regulation of starch biosynthetic genes. This suggests that light not only influences enzyme activity but also modulates the genetic pathways that govern starch biosynthesis (Kausar, 2023) [16].

The findings from this study have significant implications for sweet potato breeding and biotechnology. By understanding how temperature, light, and nutrient conditions influence starch biosynthesis, breeders can potentially select for sweet potato varieties that are more resilient to environmental stressors, such as high temperatures or nutrient deficiencies. Furthermore, genetic modification of key enzymes like AGPase and starch synthase could lead to the development of sweet potato varieties with enhanced starch yield, improved amylopectin-to-amylose ratios, or altered starch properties for industrial applications such as biofuels or food processing.

7. Conclusion

This study provides a comprehensive examination of the enzymatic pathways involved in starch biosynthesis in sweet potato (*Ipomoea batatas*) and investigates the impact of environmental factors, including temperature, light, and nutrient availability, on enzyme activity and gene expression. The results show that starch biosynthesis in sweet potato is highly regulated by developmental stages and environmental conditions. Key enzymes such as ADP-glucose pyrophosphorylase (AGPase), starch synthase, branching enzyme, and debranching enzyme exhibited the highest activity during the mid-developmental stage, which coincides with the peak of starch accumulation. Environmental factors, particularly temperature and light, were found to significantly influence the activity of these enzymes, with higher temperatures and continuous dark conditions leading to reduced enzyme activity. Additionally, nutrient availability played a crucial role in maintaining optimal starch biosynthesis, with nutrient-deficient plants showing decreased enzyme activity and lower starch production.

Gene expression analysis revealed that the transcriptional regulation of starch biosynthetic enzymes followed a similar trend to enzyme activity, with the highest expression of AGPase and starch synthase occurring during the mid and late stages of tuber development, respectively. This study highlights the importance of environmental factors in regulating starch biosynthesis at both the enzymatic and genetic levels. The findings suggest that improving environmental conditions, such as temperature management and nutrient supply, could enhance starch yield and quality in sweet potato. Furthermore, the insights gained from this study have potential implications for the development of genetically modified sweet potato varieties that are optimized for increased starch production, improved functional properties, and enhanced resilience to environmental stressors.

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