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Fruit Ripening Characterization and Amylase Mystery in Bananas

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Abstract

Bananas are well-known for their high quantities of sugar and starch, making them excellent providers of nutritional energy. The primary source of carbon storage in bananas is starch, and amylase may help mobilize starch during ripening stage. The banana-amylase activities are substantially associated with a reduction in starch content, and it is primarily up-regulated by de-novo synthesis. Studies revealed that there is a high level of transcription which results in the regaining capability for autocatalytic ethylene synthesis. Enzymatic activity varies according to fruit ripening stage and variety. The amylase status demonstrated low activity at the outset of the climacteric and a concurrent increase. Changes in cell wall hydrolase activity and starch-to-sugar conversion are linked to pulp softening and sweetening during banana ripening. To define the variations, it is critical to detect distinct enzymes such as alpha, beta, and gamma amylase, ethylene, starch, and many more. It also has an influence on the shelf life of the fruit, therefore it's important to know when and how to harvest the plant. The consistent application of ethylene or related compounds to green bananas to standardize and accelerate ripening that resembles natural maturity after increasing external ethylene output. Specific gene expression is essential for proper ripening. There is interest in using a variety of modern strategies to study fruit ripening, low-cost library screening, differential analysis, and cDNA-AFLP and high throughput microarray techniques like Real Time and Reverse Transcriptase PCR, Protein sequencer etc. The enzyme has potential uses in nearly every sector, including the detergent, textile, paper, bakery, ethanol, food, pharmaceutical production industries along with starch conversion, and agricultural waste treatment. The traditional approach used in hydrolysis of starch to glucose has many disadvantages due to the process occurring at high temperatures and acidic conditions. The current work was carried out to comprehend and define the fruit ripening phase using various morphological and molecular methodologies, as well as to know and solve the amylase enigma in bananas.

Keywords: Bananas; sugar and starch; Carbon storage; Amylase activities; Fruit ripening; Molecular screening

Introduction

The crops, fruits, and vegetables are key components of the human diet, and in recent years, numerous target-oriented scientific techniques have been established to overcome the loss caused by deadly plant and animal diseases in order to boost output by reducing loss [1,2]. Food from both animal and plant sources is essential for human nutrition. It also acts as a source of minerals, vitamin C, protein, thiamine, niacin, pyridoxine, folic acid, dietary fiber, and so on [3]. Bananas are well-known for their high

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quantities of sugar and starch, making them excellent providers of nutritional energy. The process of starch degradation in fruits such as bananas, as well as the effects of hydrolases and granule shape on starch degradation, is a hot issue [4]. The banana is a versatile fruit that may be consumed raw, ripe, or processed into a number of products. Raw bananas are frequently used in processing, whereas ripe bananas are eaten whole. The banana is a kind of tropical fruit. It is often harvested when the fruit is firm, green, and unripe [3].

The consistent application of ethylene or related compounds to green bananas to standardize and accelerate ripening mimics natural maturity after increasing external ethylene output. Pulp softening and sweetening are two of the sensory factors that impact banana pre and post-harvest quality. In the maturation process, bananas absorb a lot of carbohydrate up to 35% w/w that varies between species to species [5]. The expression of certain genes is critical during the growth and development phases of biological life [1]. One example is the de novo creation of enzymes that contribute to the autocatalytic burst of ethylene during climacteric fruit ripening [3]. Gene expression is required for several metabolic activities. Several ripening-related physiological factors have been linked to enzyme genes. Previous studies on tomato have identified the genes responsible for crucial physiological factors such as cell wall loosening, sugar buildup, color generation, and aromatic compound synthesis during ripening [6].

After an active period of cell division and cell growth, the fruit development rate slows, the ripening phase begins in Banana. As a fundamental source of energy for humans and animals, starch plays an important function in carbon-storage molecules in plants. Starch is a result of photosynthesis, which happens in plants throughout the day. The starch is broken down in response to environmental or developmental cues, such as the beginning of spring in roots and bark and the beginning of ripening in many fruits [7]. Changes in cell wall hydrolase activity and starch-to-sugar conversion are linked to softening of pulp and sweetening taste during banana fruit ripening phase. As a result, starch granule erosion and disassembly are important events in achieving optimal postharvest quality in bananas. Understanding the mechanisms of sugar primary metabolism during banana ripening is crucial for decreasing losses in pre and postharvest procedure which ultimately enhances the final quality of product. Previous studies on starch-degrading enzymes have shown that ethylene-mediated transcriptional and translational regulation is essential for sugar metabolism during banana ripening phase. Besides, ethylene's interaction with other hormones including abscisic and indole-3-acetic acid changes the metabolism of primary sugar. Studies also revealed that lot of molecular processes are involved in the regulation of metabolism throughout fruit ripening phase [5]. Despite this, several enzymes are also involved in starch breakdown. One of the most important enzymes is amylase, an endo-hydrolase capable of quickly degrading starch into soluble substrates for other enzymes to operate on. The amylase status demonstrated low activity at the outset of the climacteric and a concurrent increase. Many other fruits, including mango, have shown changes in amylase activity [8].

In higher plants, the starch is the main carbon storage form, and it is accumulated in a various organs including seeds, leaves, tubers, fruits, roots, etc. The accumulated starch is also responsible for the synthesis of soluble sugars, which gives sweetness to the fruit or plant parts. Thus, the of accumulation soluble sugar in plant parts or fruits is very important not only as a main plant strategy for the dispersal of seed through animal, but also because it impacts the quality and taste of these plant crops [9]. Furthermore, gene expression study revealed that amylases may play an important role in degradation of starch, and significantly up-regulation of amylases is associated with quicker starch breakdown in certain of the types [4].

The unripe bananas are heavy in starch, with 20-25% found in the fruit pulp. The stored polysaccharide is rapidly degraded through ripening phase, with the bulk of it being transformed into soluble sugars [3]. The hydrolytic and phosphorolysis processes appear to have a role in the course of the climacteric. In addition to ethylene, which triggers banana ripening, other hormones in plants such as gibberellic and indole-3-acetic acid can also act as starch modulators metabolism [9]. Fruit ripening is a complex phenomenon marked by major chemical changes that increase important qualitative traits such as softness, flavor, color sweetness, etc [10]. Aside from sensory qualities, the amounts of biologically active components, such as vitamins, are also altered during ripening phase that contributes to the final fruit nutritional value [11]. These modifications are highly coordinated and include numerous biochemical phases that are tightly regulated by gene expression and plant-based hormones along with epigenetic processes [12].

Ripening Phase

In the present era, we now have a better knowledge of the involvement of genes in the most vital physiological fruit changes during ripening. Identifying the genes that are active during ripening may also provide critical information on the activation of metabolic pathways and their relation to fruit quality is of utmost importance. As a result, understanding the nutritional qualities of the banana fruit throughout ripening and their relationship to the expression of important genes such as -amylase is vital for future genetic improvement projects [3]. Specific gene expression is essential for proper ripening, and one example is the de novo creation of various enzymes that are involved in the autocatalytic ethylene burst during fruit ripening of climacteric phase. Numerous other metabolic pathways, however, are also dependent on the gene regulation and expression, and specific enzyme genes have been identified as possibly regulating the most important physiological changes associated with fruit ripening such as soluble sugar

buildup, cell wall breakdown, and color production, thanks to tomato research [11].

Starch concentrations often decrease rapidly during banana fruit ripening. On average, starch content decreases from 25% in the pre-climacteric stage to around 1% in the climacteric stage. Sucrose, on the other hand, increases at a rate that is 12 times quicker than hexoses [3]. It has been revealed that breathing consumes just 5% of total energy utilized. Despite the fact that starch to sugar conversion is necessary for fruit physiology and as an edible, little is known about the molecular processes involved [12].

Genetic modification

Important data on specific significant genetic alteration in various regions can be readily utilized in genetic transformation [13]. As a result, there is interest to know and understand the fruit ripening stages by library screening, differential analysis, cDNA-AFLP and high throughput microarray techniques like Real Time and Reverse Transcriptase PCR, Protein/mRNA sequencer, Droplet PCR, Forward and Reverse Genomics etc. The majority of extant knowledge on expression of gene in ripening stages of banana and mangoes comes from the tomato as a model fruit. A high throughput examination of tomato showed a dynamic and well-coordinated expression shift in various regulatory and transcription factors, thought to be involved in the regulation of fruit ripening.

Amylase Activity

Amylase is an enzyme that hydrolyzes the alpha link of polysaccharides to break down starch into maltose and glucose. Amylase is also abundant in mammalian saliva and pancreatic juice used for food digestion, as well as in seeds as a food reserve for plant development and growth. Amylases are amylolytic enzymes that may convert starch or glycogen into useful compounds, making them an essential biocatalyst in carbohydrate metabolism [14]. Amylases are distinguished by the glycoside bond they assault. Amylases are divided into two types: endoamylases and exoamylases. Amylases are categorized into three categories based on how they hydrolyze polysaccharide bonds: α-amylases, β-amylases, and γ-amylases [15].

α -amylase

End products of the α-amylase (Enzyme Classification E.C.3.2.1.1) accelerate the breakdown of internal 1,4-glycosidic bonds of the long-chain of carbohydrates, yielding maltose, glucose, dextrin and maltotriose. These enzymes' activities are dependent on a metal cofactor, calcium [16]. The endohydrolase and exohydrolase are the two types of starch-hydrolyzing enzymes. Endo-hydrolase cleaves the amylose or amylopectin chain's internal 1-4 glycosidic bonds. On the other hand, the exo-hydrolases usually operate on the exterior glucose residues of amylose or amylopectin to hydrolyze the -1-4 glycosidic bonds or both, 1-4 and, 1-6 glycosidic bonds [15].

β -Amylase

The exo-hydrolase amylase i.e E.C.3.2.1.2 (Enzyme Classification) hydrolyzes 1, 4-glucan linkages off the polysaccharide chains non-reducing end to create various maltose subunits. Because this enzyme cannot break glycogen, branched links or amylopectin hydrolysis is incomplete. This enzyme's optimum pH range is 4.0 to 5.5. It is utilized in the distillery and brewing sectors, as well as to produce high maltose syrups [15].

γ -amylase

To produce glucose, -amylase (Enzyme Classification E.C.3.2.1.3) hydrolyzes both the -1-6 glycosidic links and the terminal -1-4 glycosidic connections. This enzyme works best in acidic settings with a pH of 3 or above [17]. Endoamylases (for example, γ-mylase) cleave an internal glycosidic link, whereas exoamylases (for example, -amylase) catalyze the breakage of glycosidic bonds towards the end of a polysaccharide [15].

Role of amalyase

During banana ripening, the degrading enzymes activity such as amylases (including α and β kinds), starch phosphorylase acid phosphatase, and oxidative enzymes such as peroxidase and catalase increased. Amylases, cellulases, starch phosphorylase, hemicellulases, sucrose phosphate synthase, Glucosidase, sucrose synthase and other enzymes have already been implicated in fruit ripening. Thus, the starch content of banana fruit at each stage is determined by three enzymes: Amylase (alpha and beta amylases), starch synthase, and starch phosphorylases, all of which play about equal roles [18].

Previous research discovered a link between amylase activity and starch concentration in fruits at three phases of maturation: tender, mature, and ripe. As demonstrated by the current study, differential genes expression involved in the synthesis of enzymes, both synthetic (starch synthase) and degrading (amylases and starch phosphorylase), plays an important role in the maturation and ripening of banana fruits [19]. Industrial applications of Amylases. The α-amylases are very important enzymes used in industrial with a wide applications range. Amylase's ability to hydrolyze the starch has made it an extensively used enzyme in a variety of sectors. The enzyme has potential uses in nearly every sector, including the paper, food, detergent, textile, bakery, pharmaceutical, ethanol production industries as well as in agricultural waste treatment [20].

The traditional acids approach of for starch hydrolysis to glucose has several disadvantages due to the process occurring in acidic conditions and also at high temperatures. These disadvantages are solved by using enzymes to produce high fructose syrup. When compared to chemical processes, the enzymatic process of starch breakdown is more environmentally friendly due to simpler process conditions and biodegradable end products [15,21].

Molecular study of amylase genes

Conventional breeding has lot of limitations like time consuming, undesirable gene recombination crossing barriers [13] but for the scarcity of knowledge on such fruits, the detection use of gene expression (especially heterologous gene sequences) throughout growth and development might offer important insights [22]. The massive sequence data amount i.e provided by whole or partial genome sequencing or gene sequencing programs and other independent laboratories can be used to identify potential candidates for further in depth, species-specific analysis on expression of various genes that are involved or take part indirectly during the growth and development phase [23].

A large number of research are conducted to produce primary access data to notice variation in the expression of various genes or cluster of genes that are possibly associated to the production phase or illness identification of plant including cereals, fruits, vegetables, etc [24]. To explore variations in expression of genes during fruit ripening phase, a restricted selection of genes is usually created in nylon membranes and probed with cDNA from mature as well as unripe fruit stages [25].

The sequences are chosen to be more closely related to the metabolic processes involved in ethylene generation, starch mobilization, cell wall deconstruction, color synthesis, and ascorbate metabolism, all of which may be important in fruit quality. Real-time PCR, reverse transcriptase (R-T) PCR, mRNA sequencing, and other techniques were used to assess the trend in amylase gene expression in numerous exotic banana cultivars during the development and ripening phases.

Methodology

Sample preparations

The banana fruit should be separated into stages for ripening and development and gathered at each step for comparative studies. Each group's fruits are stored at 20 °C in separate chambers, and sampling is done every day for respiration and ethylene tests. These fruit samples including the control and treatment groups are sliced after peeling were frozen in container filled with liquid Nitrogen at 80 °C for future examination [26, 9].

Ethylene and CO_2 emission measurements

A gas chromatograph with relevant column is often used to quantify respiration and ethylene. The fruit is sealed in jars, and after 1 hour, air samples from headspaces are usually obtained for gas analysis. The flame ionization detector is utilized for analysis of ethylene in fruits, and thermal conductivity is employed to measure CO_2 produced by breathing [27]. Calibration curves are generated in synthetic air using ethylene and CO_2 standards for comparison investigations [28, 29].

Amylase activities assay

For observing the activity of amylase in plant or fruit, the fruit samples (frozen) are crushed in to find powder with 4 vol (w/v) of 100mM phosphate buffer at pH 7.0, 1% (w/v) soluble PVP 40,000, 20mM cysteine, and1mM benzamidine. After the samples are centrifuged at 12,000g for about 40 minutes, the extracts (crude) are directly used in the experiments. The activity of amylase is measured in a 100 L reaction container containing 2U glucosidase, 36mM phosphate buffer (pH 7.0), 50 L of extract and 0.25 mol of p-nitrophenyl-maltopentaoside. The activity is measured in pnitrophenol (mg) produced per kg of total protein produced second-1 [30, 9]. The starch and soluble sugar content of banana samples are typically determined using the [9] method, whereas the protein content of crude extracts is determined using method [31, 30].

cDNA library preparation, screening, and isolation of a full-length amylase gene

Total RNA is isolated from the pulp of fruit and purified using oligo(dT) cellulose chromatography, as previously reported or with commercially available kit with manufacturer's instructions. The cDNA is usually generated and cloned into the ZAP Express vector by using previously described method [30,9] approach. After then, the cDNA library is PCR-screened for a full-length or amylase (depending on the kind of amylase) gene. The sense and antisense primers are utilized to screen the library using the sequence of an amylase clone that has been truncated at the 5 end. In contrast to the original screening technique, positive phage pools are only detected after of the PCR result that are visualized on agarose gels stained with ethidium bromide. The isolated full-length amylase clone is then excised and in vivo sequenced into the any selected phagemid [32].

Analysis and sequencing of DNA

To conduct the sequencing the DNA Sequencer/Analyzer and the Thermo Sequenase Cy5 Dye Terminator Cycle Sequencing Kit is utilized. The sequence analysis is carried out with genetic sequence analysis software package ("Wisconsin package") (GCG, Madison, WI, USA) [33]. The Clustal"X" tool is used to create the phylogenetic tree The characteristics of the deduced amino acid (a.a) sequences are acquired using the "ChloroP 1.1" [30, 9] whereas "Compute pI/Mw" algorithms, both of which are available on the Ex-Pasy service of the Swiss Institute [34].

Northern Blotting

The 10g aliquots of total RNA from banana pulp is isolated and separated on 1.5% formaldehyde-agarose gels and afterwards suction transferred to nylon membranes (Hybond N+ Amersham) as previously reported in many studies. Under normal conditions, the filters were prehybridized for 2 hours following UV crosslinking and probed for 16 hours at 65 C. [9]. The amylase probe [32P]—dCTP-random is usually primed at 1741 bp fragment from Bam HI/Xho I digestion of Ma-bmy is used for this purpose. The membranes after hybridization are washed (cleaned) at high stringency at 65

°C and is exposed for about 24 hours. The membranes for the 18S rRNA is probed, and the quantification of RNA amount is done which will be loaded on the gel lanes. Two separate northern blotting procedures were carried out [35].

Preparation of Recombinant Amylases (plant based) Expressed by Yeast Strain LL20

The selected strain LL20 is cultivated according to the technique described by Kumagai (1990) [36] to produce recombinant amylases produced by plasmid pEno/103, which includes the banana amylase cDNA fragment, OS103. The Recombinant fruit/plant amylases in culture media are precipitated with 90% (v/v) ethanol, lyophilized, diluted in a buffered solution of 100 mM Hepes-KOH (pH 7.6), 10 niM CaCl2, and 0.1% (w/v) Triton X-100, and are immune-blotted afterwards [37].

Amylase expression and production of antibodies

A segment of amylase cDNA (approx. 1155 bp in previous studies) from banana is PCR amplified using the sense and antisense primer which is cloned into the expression vector (pCRT7 /NT-TOPO in many previous studies). After the DNA sequencing, the resultant construct is utilized to convert E. coli BL-21(DE3) pLysS cells, and 1mM IPTG is employed to stimulate recombinant protein production. After 4 hours of incubation, the cells (collected) are lysed, and the 6xHys-tagged recombinant protein is extracted using Ni-column affinity chromatography. As previously disclosed, a shortened amylase about 42 kD that is encoded by the incomplete clone is validated on SDS-PAGE for visualization and is further utilized to immunize rats or rabbits for monoclonal antibodies experiment [38,39]. The antiserum is collected one week following the third vaccination dose and evaluated for reactivity against banana amylase.

Western blotting

A total of 0.5 g powdered pulp tissue extract Homogenizing in 2mL of commercially available buffer yielded the protein extracts. A total of 50 g protein extracts is loaded in each well for separation with SDS-PAGE and transferred to nitrocellulose after boiling for 10 minutes. After then the filters are probed are diluted 100th times with amylase antibody and are then treated with 8000 times diluted anti-rabbit goat IgG that is linked to horseradish peroxidase [9]. The cheiluminescent detection with luminol is used to view the reactive protein. Replicate trials should be conducted for authenticity.

DNA extraction

DNA extraction is usually done by CTAB method or commercially available kit by following manual instruction and Polymerase Chain Reaction is carried out by the previously reported protocol [1,22]. The extracted DNA for the samples can be used for variety of advanced technique like whole or partial genome sequencing, Real Time or Reverse Transcriptase PCR [1].

Total RNA Extraction

Total RNA is extracted from the fruits 8 days after treatment using the improved CTAB technique and then treated for 30 minutes with RNase-free DNase I to eliminate genomic DNA contamination. A NanoDrop 1000 spectrophotometer and an Agilent 2100 bioanalyzer were used to determine the concentration and integrity of each RNA sample. Samples having concentrations more than 400 ng/L, RIN (RNA integrity number) values greater than 8, and OD of 260/280 and 260/230 ratios greater than 1.8 were chosen for library creation. The Beijing Genomics Institute (BGI, Shenzhen, China) used an Illumina HiSeqTM 2000 platform to build and sequence libraries. For each sample, three distinct libraries were sequenced as biological replicates [40].

Gene Selection and Primer Designing

The nucleotide coding sequences of the selected -amylase (GenBank: AF533648.1) and Actin (GenBank: AB022041.1) genes were retrieved from the National Center for Biotechnology Information (NCBI) database. The actin gene is used as an internal control in this study. Forward and reverse primers were created from the coding sequences of the aforementioned genes using the Primer 3 online application.

1.1.1. **Determination of starch and sugar contents:** The starch content of banana samples are determined using the acid hydrolysis method. The samples are hydrolyzed in the presence of water and HCl to turn all of the starch into sugars. The converted sugar is then examined using the Lane and Eynon procedure described in to measure the starch concentration, which is then multiplied by a starch factor (0.90). Sugar is extracted using a water-ethanol (1:1) mixture, the ethanol is allowed to evaporate, and the sample are diluted to a level of 250 ml with distilled water before being neutralized. After that, the Fehling's solution is titrated with the sample solution until a brick red hue developed.

Real-Time Quantitative RT-PCR

The transcripts of chosen candidate genes associated to starch and sucrose metabolism were quantified using real-time quantitative RT-PCR (qRT-PCR) with cDNA produced from RNA samples of banana fruits at ripening stages 1, 3, 5, and 7 using the ProtoScript® First Strand cDNA Synthesis Kit (NEB, Ipswich, MA, USA). In qRT-PCR, primer sequences are employed. The relative expression level is determined using the 2Ct formula. The potential genes' expression levels in Ct values were first normalized to ACTIN expression levels, and subsequently to the corresponding expression levels in WT plants. There were three replicates in each experiment. The relative mRNA abundances were shown using heat maps [41].

Amylase Transcripts exhibit variable expression at different Developmental and Ripening Stages

The banana fruit during ripening is chosen for amylase

expression studies and its relationship to sugar accumulation because the ripening stages in banana are easily identified, and secondly, the banana is thought to contain high sugar and is a good candidate for the study of amylase expression and sugar accumulation during ripening. RT-PCR is performed to examine the expression patterns of amylase gene transcripts in banana cultivars at various stages of fruiting [3].

The expression level of the -amylase gene transcripts fluctuate dramatically during the development and ripening stages of Williams', according to studies. Transcription of the gene begins at pre-climacteric stage 4. Prior to this, the transcript signals remain undetectable, i.e., the first three stages. The intensity of the amplified products increases steadily from the middle to the end of the cycle, suggesting that RNA accumulation (gene expression) is growing. The -amylase gene has the highest levels of expression at stage 6, as indicated by the bright bars [3].

This might indicate the ripening stage, when starch breakdown is at its maximum. Following this peak, there is a constant pattern of gene expression throughout the successive stages of ripening, indicating a gradual reduction in starch content. Amylase gene expression is greater during fruit ripening phases than throughout other developmental stages, according to the studies described above. The general pattern of gene expression in all similar species (with common parent) is usually identical with slight differences [42]. The abundance of mRNA is seen on day 2 in ethylene-treated fruit, whereas the peak of transcripts are observed on day 16 in control fruit, and transcript accumulation in fruits is delayed by 24 days [43]. Several studies found comparable outcomes with the amylase gene. It is observed that the accumulation of sugars and organic acids alters the aroma and flavor of tomato fruits. Throughout fruit ripening, starch is converted to sugars, affecting the flavor and, ultimately, the quality of mature tomato fruits [44]. The amylases, a group of essential starch hydrolytic enzymes involved in starch breakdown, were examined in cherry tomato cultivation. The accumulation of gene transcripts at the plastidial isoenzymes is linked to delayed activation of the amylases enzyme during tomato fruit growth and maturity, which contributed to starch depletion and an increase in total soluble solids [44].

Total starch content measurement

To prevent browning, the pulp extracted from fruit (banana) is soaked for 10 minutes in a 1.5% sodium bisulfite solution before drying for 24 hours at 40 °C and being milled into fine powder. A total of 100mg of fine powder is then washed in 5 mL of 80% ethanol and centrifuged at 4000-5000 rpm for 5-6 minutes. After removing the supernatant, the pellet is thoroughly washed thrice in 5 mL of 80% Ca $(NO3)_2$ and centrifuged for 5-6 minutes at 4000-5000 rpm. The total starch content is estimated as a percentage per gram of dry material by using a 100 g/mL starch standard solution with an absorbance of 620 nm. Three duplicates are usually taken for correct reading [45].

Heat Map Illustration by R software

The graphical representation of gene expression patterns related with amylase, ethylene, glucose and sucrose metabolism, enzymes with mystery in fruit ripening, etc are usually displayed as heat maps using the R programming language with packages like G-plots, GGE, PCA, PCOA [1]. The number of final assembled contig IDs in unigene libraries from transcriptome sequencing following RNA-Seq are observed. Furthermore, the BlastN is usually used to generate heat maps for IDs that explicitly matched to identified genes related to any parameter including characterization or involvement of genes in fruit ripening in the fruit (for example Banana) Genome Hub, along with pairwise sequence comparisons over 95% probability [41].

Discussion

The yield or diseases related to both plants and animals are economically significant and increase in yield and control and eradication of diseases are top priorities, and the only way for effective control is long term fertility building, which requires a combined effort and methodology for targeted way-out instead of individual and short-term approach in conventional programs [1,2]. Recent advances in methods, such as development Nanotechnology (testing on vero cell lines), genetic modification, transformation, enzyme investigations and application, and so forth, are regarded as valuable assets in boosting yield [38].

Furthermore, treatment must be carried out according to the stage of the plant in order to enhance output while minimizing costs, which may be performed through a variety of approaches [38,1]. A common example is the usage of ethylene in banana ripening. The transfer of carbon from starch molecules to soluble sugars is one of the most critical metabolic shifts in banana ripening (different stages), along with peak ethylene production and a respiration spike. Because a significant conversion happens in a less time duration, it indicates an effective catalytic system, that contribute in making a great model of fruit (for example banana) for studying the enzymes (amylase, etc) and other parameters that regulate the starch breakdown process in fruit [3].

Ethylene is known to be a fruit ripening trigger; however, the process of starch breakdown is yet to be explored. One of the reasons may be that it occupies ethylene receptors and suppresses ethylene perception in several fruits [46]. Study have also reported that the expression of beta-amylase is considerably enhanced during ripening phase of banana, and the rise in activity is highly associated with starch breakdown [47].

[48] Dapeng and Yongzhang (2002) presented the first description of amylase subcellular localization in fruits from apple pulp cells by detecting the enzyme near the peripheries of starch in amyloplasts. Previous studies has deduced amylases a.a sequences by revealing that the enzyme from fruit bananas (Ma-bmy) might be grouped with the isoenzymes from rice (Os-bmy), *Prunus*

armeniaca (Pa-amyb) and Arabidopsis (At-bmy3). Furthermore, it is probable that this group of amylases shares certain features as well as a distinct physiological purpose.

The rise in amylase activity is caused by de-novo production of the bananas enzyme that is exposed to ethylene during normal phases of ripening. In previous studies it was reported that the quantity of protein and amylase activity in fruit treated with the "Monocyte Chemoattractant Protein" (1-MCP) is nearly undetectable throughout the trial, despite a substantial increase of transcription afterwards, as a result of the apparent recovery of ethylene synthesis. There is a considerable lag between the polygalacturonase gene transcription and accumulation of protein in transgenic tomatoes with decreased ethylene production [49,9].

Various investigations also found that ethylene facilitated transcription and translation, although the sensitivity thresholds for these activities varied. Furthermore, these findings give preliminary data on the control of amylase expression in bananas. Many research have focused on the ethylene hormone because of its critical function in controlling climacteric fruit ripening and fruit senescence, among other things. These processes can be inhibited by using 1-MCP, which binds strongly to ethylene receptors and thereby blocks the ethylene signaling pathway. This protein has been shown to block ethylene activity and delay the climacteric ripening of tomato fruit. Moreover the enzyme activity is largely controlled by expression of required gene, and the lack of amylase protein in 1-MCP treated fruit samples suggests that translation is also controlled by ethylene [50].

Studies have also reported that it is unclear if differing ethylene thresholds would influence expression of amylase at both translation as well as transcription level. It is revealed that even if a link among amylase activity and starch regulation can be established, degradation can occur gradually without any increase (extra) in the enzymes' pre-existing activity or expression [9].

Mature green banana fruit can contain up to 20% starch, which is destroyed throughout the ripening process by alpha-amylases (alpha-1,4 and alpha-1,6-glucosidases), beta-amylases, and phosphorylases that compete for the same substrates [51]. The study of gene expression also revealed that amylases play an important role in starch degradation in banana species, and that highly up-regulated amylases are also associated with a quicker rate of starch breakdown process. Study has also revealed that two amylases, one starch phosphorylase, and one starch debranching enzyme are specifically up regulated, suggesting that they may hydrolyze more non-resistant starch (RS) in most of the varieties [4].

Conclusions and Recomendations

Bananas are well-known for their high sugar and starch content, making them great sources of nutritional energy. Starch is the principal source of carbon storage in bananas, and amylase may aid in starch mobilization throughout the ripening period. The activities of banana-amylase are significantly related with a decrease in starch content, and it is predominantly up-regulated by de-novo synthesis. The amylase status showed low activity at the start of the climacteric and a rise concurrently. During banana ripening, changes in cell wall hydrolase activity and starch-to-sugar conversion are connected to pulp softening and sweetening. To characterize the variances, various enzymes such as alpha, beta, and gamma amylase, ethylene, starch, and many others must be detected. It also affects the shelf life of the fruit, so knowing when and how to harvest the plant is critical. Fruit ripening stage and variety influence enzymatic activity specially amylase. Many studuies revealed that transcript expression for the amylase gene is lower in the early stages but increases with the passage of time as the fruit ripens. Similarly, during the ripening stage, there is a significant decrease in starch concentration across the board, but there is also a commensurate increase in sugar content due to starch breakdown, but it strongly depends on the variety used as slight to high variation may exist. Controlling sugar accumulation and amylase role at a certain developmental stage also benefit from expression analysis, so more studies must be done to explore amylase further and also its role, association, correlation with other enzyme for accurate prediction fruit harvest with maximum taste and nutrients.

Acknowledgement

None.

Conflict of Interest

None.

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