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Impact of exogenous pyridoxin treatments of 'Williams' banana on the activities of starch degrading enzymes

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ABSTRACT

Pyridoxine activates many different enzymes in the plant cell and it contributes to many various physiological processes. It has been examined for stimulating and enhancing banana fruit 'Williams' (Giant Cavendish AAA sub-gathering) during12 days of the shelf life. Many different concentrations of vitamin B6 0, 3, 6, 9, and 12 mM have been applied to bananas for 30 min. Thereafter, fruit stored at room temperature (25 ± 1oC and air humidity average 56 ± 2%) for twelve days. Fruit samples were selected each 3-day intervals. The physical and chemical analysis was determined to evaluate fruit ripening quality elements. The physical quality attributes of fruit were measured (brown skin spot index and color hue angle). Additionally, the chemical properties determinations were investigated such as starch content and starch catalyst breakdown activities, for example, a-amylase (AMY; EC: 3.2.1.1), phosphorylase (PHO; EC: 2.4.1.14), and invertase (INV; EC: 3.2.1.26). The sugar profile (reducing, non-reducing, and total sugar content) was estimated. In term of fruit quality parameter, peel fruit firmness, degradation of chlorophyll, and ß-carotene content were measured during the shelf life period. Pyridoxine treatment at 9 mM gives a higher starch breakdown and total soluble sugars. Moreover, increasing the ß-carotene content of fruit peel which reflects on the color hue angle of fruit. While the decreases in both total chlorophyll and phenol content were observed after treating by Vit. B6 during the shelf-life period. Also, fruit presented a normal ripening proprieties and less ripening fruit spotting index under moderate browning symptoms index 12th day. It was concluded that pyridoxine application at 9 mM has generative effects on fruit ripening and optimize the quality of 'Williams' banana at room temperature to normal ripening.

1. Introduction

Banana is a climacteric natural product that shows numerous morphological and physiological changes in the substance of fruits during ripening stage. These progressions are firmly connected with respiration and ethylene processes (Palomer et al., 2005). For the most part, banana collected in an extensive variety of physiological stage, from half to fully developed and a brilliant quality after ethylene application in ripening store (Klieber et al., 2002). Economically, bananas reaped at the green color stage and, ripening is initiated by vaporous ethylene (Botondi et al., 2014). Numerous postharvest systems have been implemented on a banana to keep up their quality and extend storage capacity life, for example, ethylene treatment (Botondi et al., 2014), utilizing heat treatment on 'Sucrier' banana cv. (Kamdee et al., 2009), controlling banana aging by N_2O (Palomer et al., 2005), using GA3 and benzyl adenine on two banana verities in light of various postharvest treatments keeping in mind the end goal to keep up banana fruits quality and increment shelf life period (Zomo et al., 2014). Other different studies utilized edible coating for increasing shelf life period, for example, chitosan as coating treatment was applied to 'Cavendish' banana fruits (Suseno et al., 2014).

Vitamin B6 (Vit.B6) constitutes a gathering of exacerbates that are engaged with a shockingly high assorted variety of biochemical response in a plant cell (Mooney and Hellmann, 2010). There are three essential types of water-dissolvable vitamin B6: pyridoxine (PN), pyridoxal phosphate (PP) and pyridoxamine (PM). These forms are found in an assortment of plant tissue of horticultural commodities (Kannan and Jain, 2004). Also, all forms are subject to phosphorylation processes. Many researchers investigated the roles of PN in the plant. Plants catalyze PN in the cell though, pyridoxal 5'-phosphate (PLP) pathway which it appears strongly active molecule and normally used as a cofactor for enzymatic reactions (Mooney and Hellmann, 2010).

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Meanwhile, it is initiated in excess of 140 various enzymatic responses (Percudani and Peracchi, 2009). PLP has specifically incorporated two complex proteins which are PDX1 and PDX2. Two pathways to amalgamation in the plant cell, one is broadly distributed in nature, in both animals and plants. While, the new pathway called 'Rescue pathway' has been accounted for in various plants (Huang et al., 2013). Likewise, it has a part in ensuring plant cells against oxidative reactions by responding as a quencher of active oxygen species and filling in as a cofactor to ascorbic acid and α-tocopherol by which it secures the plasma cell membrane functions (Huang et al., 2013). Moreover, it is biosynthesis and catalyzed amino acids, and it involved in starch hydrolysis (Mooney and Hellmann, 2010). Starch breakdown includes an underlying advance the action of PLP-subordinate chemical α-glucan phosphorylase, which catalyzes glucose-1-phosphate from the non-diminishing end of α-1,4-connected glucan chains (Zeeman and Smith, 2004). Glucosinolate assistants are a gathering of aggravates that are involved with plant safeguard (Grubb and Abel, 2006). Likewise, it impacts on biosynthesis auxin which is required for plant advancement (Tao et al., 2008), and ethylene biosynthesis and debasement in the two procedures (McDonnell et al., 2009).

The main goal is of this study is determining the impact of Vitamin B6 on starch degradation enzymes while forcing banana fruits towards maturation stage after harvesting.

2. Materials and methods

2.1. Plant materials and experimental setup

Pre-climacteric green banana 'Williams' fruit (Giant Cavendish AAA sub-group) grown in clay loam soil in a commercial orchard in Meet Ghamr, Dakahlia perviness (latitude 30.71 and longitude 31.25), Egypt. The experiment was conducted during two seasons 2016 and 2017 at first and second ratoon. Banana hand (Hands are several bananas on a stem pulled from the stalk) samples (75) were selected for uniformity of size and color and cleaned in a solution of 200 $\mu L\,L^{-1}$ chlorine. Afterward, fruits were then dipped for 2 min in 2 $mg\,L^{-1}$ Benlate® solution to control fruit rots and there were allowed to dry before treatment at room temperature. Banana samples were transported in cold track at 15 °C for 3 h. Upon arrival to the lab, fruit samples were immunized 30 min in dark at room temperature in a solution of pyridoxine at different concentrations 0, 3, 6, 9, and 12 mM to stimulate fruit ripening. Vitamin B_6 or Pyridoxine (169.18 molecular weight) is water-soluble vitamin and it was purchased from El-Gomhoria Co. suppliers, EGY, the purity was 98%. Each treatment has fifteen hands, five for color measurement and ten for chemical analysis. Fruits were stored for twelve days at room temperature (25 ± 1 °C and air humidity average during the shelf-life period 56 ± 2%). Pyridoxine treatment at 0 Mm is considered as a control (fruits were immersed in water) and all treatments were applied at room temperature in dark conditions.

2.2. Starch content determination

Fruit pulp samples 2 g were mixed with 5 mL diethyl ether, and the soluble sugars were excluded three times using ethanol 80% at 80 °C by 5 mL, then samples were dried at 70 °C, and the residue (starch) was dissolved with 0.5 mL of 0.25 M of H_2SO_4 at 100 °C for one hour. The solution was mixed with 0.5 mL of 9,10-dihydro-9-oxoanthracene (anthrone) under acidic condition (0.1 g 100 mL^{-1} of 76% H_2SO_4). Then the mixture was heated for 10 min and cooled (Stevens and Chapman, 1955). The blank sample was prepared with distilled water. The absorbance was determined at 620 nm in spectrophotometer UV–vis auto Model, LaboMed, Inc USA. The concentration of glucose was figured from the standard bend $Y = 0.0026\,X + 0.0123$, where Y = absorbance (test – clear) and X = glucose concentration ($R^2 = 0.9981$). The centralization of starch substance was estimated by increasing the multiplying got for glucose by the transformation factor 0.9.

2.3. Enzyme extraction and assay

Banana pulp sample (1 g) was added in 5 mL of a cradle containing 50 mM Hepes-NaOH cushion (pH 7.5), 20 mM $MgCl_2$, 10 mM ascorbic acid, 1 mM EDTA, 10 mM β-mercaptoethanol and 2% (w/v) insoluble polyvinylpyrrolidone. All means amid extraction and measure were performed at 0– 4oC.

α-amylase (AMY, EC: 3.2.1.1) measure was completed by a change of methods (Nielsen et al., 1997). Protein extricate 0.5 mL was blended with 0.5 mL of Mes-KOH (pH 6.2), and the response was begun by including p-nitropheylmaltoheptaoside as substrate. The response was ceased by including 0.25 mL of 10% trizma base and the action decided as freed p-nitrophenolate distinguished spectrophotometrically at 405 nm.

Invertase (INV, EC: 3.2.1.26) action, 0.5 mL protein remove was hatched for 45 min at 25oC with 0.5 mL of 50 mM Mes (pH 5.5) containing 50 mM sucrose, and after that 2 M NaOH was added to the measure blend to stop the response. Hexoses shaped after hatching were measured by the dinitro-salicylic acid test (Chaplin, 1986).

Phosphorylase (PHO, EC: 2.4.1.14) action was estimated by the development of UDP amid the amalgamation of sucrose-6-P from fructose-6-P and UDP-glucose. UDP was tested as the utilization of NADH within the sight of pyruvate kinase and L-lactate dehydrogenase (Van huylenbroeck and Riek, 1995).

2.4. Sugars profile

Sugars were controlled by Inverting sugar decreases the copper in Fehling's answer for red, insoluble cuprous oxide. The sugar substance in banana mash tests was assessed by deciding the volume of the obscure sugar arrangement required to totally decrease a deliberate volume of Fehling's answer. Before utilizing, the blend [1:1] of Fehling's answer An and B (5 mL of each) was institutionalized against standard glucose for getting glucose identical and to land at a change factor (Paramesha et al., 2017).

Reducing Sugars: the 2 g natural product mash was taken in a volumetric cup with 2 mL of 45% fundamental lead acetic acid derivation arrangement was included for elucidation. Following 10 min at room temperature, the arrangement was weakened by including potassium oxalate gems in abundance and the volume was made up to a known sum with refined water and separated through Whatman No. 1 channel paper. The filtrate was taken in a burette and titrated against heating up Fehling's blend (5 mL of Fehling's answer A + Fehling's answer 5 mL of B) till the blue shading blurred. At that point, one mL of methylene blue marker (1%) was included and the titration has proceeded until the substance achieved a block red shading and titer esteem was noted. The percent of diminishing sugars was ascertained (Prout et al., 1971). For estimation of total sugars, the filtrate acquired during reducing sugars estimation was utilized. An aliquot from the filtrate was taken and to the one-fifth of its volume, hydrochloric corrosive (1:1) was included and the reversal was done at room temperature for 24 h. Consequently, the substance was cooled and killed with 40% NaOH utilizing phenolphthalein as a pointer and the last volume was made up to 100 mL. The total sugars were expressed as a percentage in terms of invert sugars. The aggregate sugars were communicated as a rate as far as rearrange sugars. The non-reducing sugars in rate were figured by increasing the distinctions of total and reducing sugars by a factor of 0.95. The outcomes were communicated as a percent.

2.5. Physical and chemical quality analysis

Color hue angle (h^o) was determined on the banana fruits peel in time intervals during the experiment (Lo'ay and El Khateeb, 2011). Thereafter, all images were analyzed by using the software package ImageJ Ver. 1.43 u USA to get the RGB signals to compute shade point of bunches as per (Khojastehnazhand et al., 2010).