



Research Article

Effect of *Fusarium musae* on cell wall degrading enzymes, total soluble sugar and total phenol content of infected banana fruits

T. T Baria, K. B. Rakholiya

Abstract

In this study, the effect of inoculation of *Fusarium musae* on ripe and unripe banana fruits was observed. Phenylalanine ammonia lyase (PAL) activity was higher in unripe inoculated fruits ($73.22 \text{ U h}^{-1} \text{ g}^{-1} \text{ protein}$) than uninoculated ($58.42 \text{ U h}^{-1} \text{ g}^{-1} \text{ protein}$), while it decreased in ripe inoculated ($9.15 \text{ U h}^{-1} \text{ g}^{-1} \text{ protein}$) and uninoculated fruits ($3.35 \text{ U h}^{-1} \text{ g}^{-1} \text{ protein}$) after 72 h of storage. Polyphenol oxidase (PPO) activity of uninoculated ($2.3 \text{ U min}^{-1} \text{ g}^{-1} \text{ protein}$) was more or less similar to inoculated ($3.0 \text{ U min}^{-1} \text{ g}^{-1} \text{ protein}$) fruits after 72 h of storage. However, in uninoculated and inoculated semi-ripe fruits, PPO activity was increased about 16.0 folds and 18 folds after 72 h incubation, respectively. At the 0 h incubation the PPO activity was 4.61 fold higher in ripe fruits as compared to semi-ripe fruits, but it was increased after 72 h in both inoculated and uninoculated semi-ripe fruits. Peroxidase (POX) activity was higher in ripe fruits ($48.85\text{--}120.9 \mu\text{mol guaiacol min}^{-1} \text{ g}^{-1} \text{ protein}$) than in semi-ripe fruits ($8.65\text{--}60.9 \mu\text{mol guaiacol min}^{-1} \text{ g}^{-1} \text{ protein}$) up to 72 h of storage. The POX activity was enhanced after inoculation with *F. musae* in all ripening stages. The degree of increase in infected unripe, semi-ripe and ripe was 11.71, 7.0 and 2.47 fold higher respectively than uninoculated fruits. Total soluble sugar content was higher (208.65 mg/g fresh weight) in ripe uninoculated fruits and still further decreases (195.87 mg/g fresh weight) in inoculated fruits and lowest in uninoculated unripe fruits after 72 h of storage. 9.22 fold increases in total soluble sugar content was observed in unripe inoculated fruits followed by unripe uninoculated fruits and semi-ripe fruits. Total phenol content was higher in unripe fruits (15.71 mg/g fruit weight) than semi-ripe (13.49 mg/g fruit weight) and ripe fruits (10.59 mg/g fruit weight) at initial time. The phenol content was decreased with increasing storage period in uninoculated fruits and further decreased in inoculated fruits in all three stages.

Keywords *Fusarium musae*, PAL, POX, PPO, total phenol, total soluble sugar

Introduction

Banana (*Musa paradisiaca* L.) is one of the most important commercial fruits crop grown all over the world in the tropical and subtropical areas. It is the second-largest fruit crop in India, belongs to the family *Musaceae* in order *Zingiberales*. It is the most important fruit crop of the south Gujarat region. The farmers prefer it because of its high demand for fresh fruit in the market. In India, the banana is the fourth important food crop in terms of gross value exceeding only paddy, wheat, and milk products. It is also a desert fruit

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for million apart from a staple food owing to its rich and easy digestibility. The ripe fruits are edible, delicious, and very nutritious. The content of carbohydrates 22.84 g is very high with a calorific value of 89 kcal/100 g fruit. It is a good source of vitamin A (64 IU/100g of edible portion) and vitamin C (8.7mg/100g of edible portion) and a fair source of vitamin B₁, B₂, B₃, B₅, B₆, and B₉. The fruits are rich in magnesium, sodium, potassium, and phosphorus. The food value is about three times that of wheat. It makes a healthy and salt-free balanced diet than many fruits. Banana fruit powder made from dried green fruits can be used as baby food and in the manufacture of chocolate and biscuits. The ripe fruits are used to prepare wine, jams, jellies, puddings, and halwa. The banana plant produces the parthenocarpic fruit of commercial importance and propagated vegetatively from underground storage organ (rhizome or sucker) and surface level is the meristematic region pseudostem which gives rise to the leaves and finally to the inflorescence which produces the fruit. The leaves emerge in sequence with each rolled leaf pushing throughout the center of an increasingly greater number of over-lapping leaf sheath bases which constitute a pseudostem. The pseudostem produces flowers only once and it cut off after fruiting. The fruits are called fingers, which are borne in hands. The usual practice is to harvest a banana before it is ripe. The ripening is then done artificially in various ways, such as exposing the bunches to the sun, placing them over a hearth, wrapping them with green leaves, and piling them in a heap, storing them in closed godowns or smoking them, or treated with ethylene. The fruit takes 48 to 72 h to ripe [1].

Fusarium musae caused through contact with *Fusarium musae* contaminated banana fruits, either being imported or after traveling of the patient to a banana producing country. An alternative hypothesis is that *Fusarium musae* is not only present on banana fruits, but also other plant hosts or environmental sources. In a more recent survey performed laboratory testing the feasibility of an in house developed MALDI-TOF MS identification assay and thereby using 390 fungal isolates collected between July 2012 and July 2013 from 2 hospitals located in Brussels, one *Fusarium musae* strain was found among the 20 *Fusarium* isolates identified. This *Fusarium musae* strain was isolated from a blood sample of an immune-suppressed patient, whereas the majority of the other fusarioses [2].

Very meager research work has been done on fusarium fruit rot diseases of banana and their management in India, therefore to extend the shelf life of banana fruits and to reduce the losses caused by post-harvest diseases; it is felt worthwhile to carry out the investigations on fusarium fruit rot diseases of banana under south Gujarat condition.

Methodology

Studies on cell wall degrading enzymes

Unripe, semi-ripe and ripe fruits were surface sterilized and separately inoculated with *Fusarium musae* by the pin-prick method. The inoculated fruits were incubated at ambient temperature. The extracts from inoculated and uninoculated unripe, semi-ripe and ripe fruits were collected after 0, 48, and 72 h. inoculation.

Phenylalanine ammonia-lyase (PAL) activity

Three hundred milligrams of fruit tissue homogenize with a pre-chilled mortar and pestle in 3ml of extraction buffer containing 50 mM borate-HCL buffer (pH 8.5) and 0.04 percent β -mercaptoethanol. The homogenate was centrifuged at 10,000rpm for 15min. at 4°C. The clear supernatant was used as the enzyme source for the assay of Phenylalanine ammonia-lyase [3].

The reaction mixture containing 3.0ml of 0.1M sodium borate buffer (pH 8.8), 0.5ml of 0.1M phenylalanine (dissolved in 0.1M sodium borate buffer, pH 8.8). The reaction was initiate by the addition of 0.1ml enzyme extract. The tubes were incubated at 37°C for 2 h. The blank was also set with the substrate (containing 3.0ml of 0.1M sodium borate buffer, pH 8.8, and 0.5ml of 0.1M phenylalanine). The O.D. reading at 290nm after 2 h. The enzyme activity was expressed as U/hr/g protein.

Polyphenol oxidase (PPO) activity

Three hundred milligrams of fruit tissue grounding with a pre-chilled mortar and pestle in 3ml of 0.1M sodium phosphate buffer, pH 6.0. The homogenate was centrifuged at 10,000rpm for 15min. at 4°C. The clear supernatant was used for enzyme assay. The reaction mixture contains 2.9ml of catechol (0.01M catechol in 10mM phosphate buffer, pH 6.0) and the reaction was initiate by the addition of 0.1ml enzyme extract. The changes in the color were due to the oxidized catechol was reading at 490nm for one minute at an interval of 15seconds. The enzyme activity was expressed as a change in O.D./min/g protein [4].

Peroxidase (POX) activity

The banana fruit tissue samples (1g) were homogenized with a pre-chilled mortar and pestle in 2ml of 0.1M phosphate buffer, pH 7.0 at 4°C. The homogenates were centrifuged at 12,000rpm at 4°C for 15 min. and the supernatant was used as an enzyme source. The reaction mixture consists 2ml of 50mM phosphate buffer (pH 7.0) and 0.1mM EDTA, 10mM guaiacol 450μl, 10mM H₂O₂ 450μl and 100μl enzyme extract. POX activity was determined in the homogenates by measuring the changes in absorbance at 420nm due to the formation of tetraguaiacol ($\epsilon = 26.6\text{mM}^{-1}\text{cm}^{-1}$) in a reaction mixture was recorded at 15seconds intervals for 1minute. The enzyme activity was expressed as changes in the absorbance of the reaction mixture min-1g-1 on the fresh weight basis [5].

Studies on total soluble sugar and total phenol content***Total soluble sugar content***

Total soluble sugar from the semi-ripe banana pulp both inoculated and uninoculated determine by homogenized pulp and pericarp (100mg) was extracted with 5ml of 80 percent ethanol and centrifuge at 3000rpm for 10minutes. Extraction was repeating 3 times with 80 percent ethanol and supernatants collect into 25ml volumetric flasks. The final volume of the extract was made to 25ml with 80 percent ethanol. The extract (0.3ml) was pipetting from each treatment into separate test tubes and the tube was placed in a boiling water bath for 3 minutes to evaporate the ethanol. One ml of MillQ water and 4ml of 0.2 percent anthrone reagent (200 mg in 100ml H₂SO₄) was added in each test tube and place in ice-cold water. Reagent blank prepared by adding 1 ml of distilled water and 4ml of anthrone reagent. The intensity of color was reading at 600nm on a spectrophotometer. A standard curve was prepared using 10mg glucose per 100ml distilled water [6].

$$\text{Total soluble sugar (mg/g)} = \text{Sample O.D.} \times \text{Standard O.D.} \times \text{Dilution factor}$$

Extraction and estimation of phenolic compounds

Total Phenol from the semi-ripe banana pulp both inoculated and uninoculated determine by One gram of fruit tissue homogenize with a pre-chilled mortar and pestle in 10ml of 80 percent methanol and the extracts left for 24hr at room temperature before centrifuging at 15,000rpm for 10minutes [7]. One ml methanolic extract add to 5ml of distilled water and 250μL of Folin ciocalteu reagent and the solution was kept at 25°C for 3min. Then 1ml of a saturated solution of Na₂CO₃ (20per cent solution of Na₂CO₃) and 1 ml of distilled water add and the mixture was incubated for 1hr. at 25°C. The absorption of the developed blue color measured using a spectrophotometer at 725nm of a single wavelength. The total phenol content was calculated by comparison with a standard curve obtained from using pyrocatechol ranging between 0-25μg concentration. The amount of phenols present in the sample was calculated by the following formula:

$$\text{Phenol (mg/g)} = \text{Sample O.D.} \times \text{Standard O.D.} \times \text{Dilution factor}$$

Table 1. Biochemical changes in banana fruits infected by *Fusarium musae*

PAL Activity U/hr/g fruit weight								
Sr. No.	Sample	0 h.	48 h. after		72 h. after		Fold	
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
1	Unripe	50.445	18.235	2.647	58.419	73.216	+1.15	+1.45
2	Semi-ripe	7.545	6.211	11.371	60.548	48.423	+8.0	+6.41
3	Ripe	12.564	4.504	9.305	3.347	9.151	+3.75	+1.37
PPO Activity U/min/g fruit weight								
Sr. No.	Sample	0 h.	48 h. after		72 h. after		Fold	
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
1	Unripe	0.275	0.327	0.680	2.257	3.05	+8.2	+11.0
2	Semi-ripe	0.358	0.431	0.735	5.751	6.471	+16.0	+18.0
3	Ripe	1.652	2.064	2.501	3.674	4.384	+2.22	+2.65
POX Activity U/min/g fruit weight								
Sr. No.	Sample	0 h.	48 h. after		72 h. after		Fold	
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
1	Unripe	3.520	16.574	28.681	25.585	41.234	+7.27	+11.71
2	Semi-ripe	8.645	38.523	36.341	51.741	60.884	+5.99	+7.0
3	Ripe	48.845	64.631	92.243	109.85	120.85	+2.25	+2.47

Mean of three replication, *Change over time in uninoculated and **Change over time in uninoculated on inoculated

Results and Discussion

Studies on cell wall degrading enzymes on disease development

Quality is one of the most important factors in horticultural crops, which determine the nutrient content in fruit as well as its shelf life, so it is necessary or to find out the different biochemical factors that are associated with fruit of banana so that present investigation carried out on banana fusarium fruit rot incited by *Fusarium musae*. In this study, the changes in the levels of total phenol and total sugar as well as the activities of phenylalanine ammonia-lyase (PAL), polyphenol oxidase (PPO) and peroxidase (POD) enzymes during banana fusarium fruit rot infection and ripening were determined. Pulp with pericarp samples from healthy and rotted fruits was collected at three ripening stages i.e., unripe, semi-ripe, and ripe fruit with and without inoculated at respective stages and changes in biochemical were studied after 0, 48, and 72 h. The samples were either used immediately or kept frozen in liquid nitrogen and stored -20°C until use for analysis of metabolites constituents about disease development.

Phenylalanine ammonia-lyase (PAL) activity

The results demonstrated in table 1 that enzyme activity was higher in unripe inoculated fruits (73.22 U h⁻¹ g⁻¹ protein) after 72 h than uninoculated (58.42 U h⁻¹ g⁻¹ protein) which was decreased in ripe inoculated fruits (9.15 U h⁻¹ g⁻¹ protein) and uninoculated (3.35 U h⁻¹ g⁻¹ protein) after 72 h of storage. These results suggest that the constitutive level of PAL was higher in unripe fruits then decreased with the ripening stages. It was first decreased then increased in inoculated unripe fruits. The PAL activity was increased after 24 and 72 h storage in unripe and semi-ripe fruits irrespective of infection, while decreased in ripe fruits as compared to 0 h. However, PAL activity was 1.37 fold higher in inoculated ripe fruits as compared to uninoculated ripe fruits at 72 h incubation. This suggested that during ripening in healthy fruits phenol synthesis is decreased.

These results are corroborated with earlier findings of Nath et al., [8], who indicated that the constitutive level of PAL was higher in green mature banana fruits then decreased with the ripening stages. It was first decreased then increased in inoculated green mature fruits. The PAL activity was increased after 24 and 72 h storage in green mature and semi-ripe fruits irrespective of infection, while decreased in ripe fruits as compared to 0 h. However, PAL activity was 2.5 fold higher in inoculated ripe fruits as compared

to uninoculated ripe fruits at 72 h incubation. A similar result was observed by Patel [9] in banana fruit infected by *Colletotrichum gloeosporioides*.

Polyphenol oxidase (PPO) activity

The PPO activity was found (Table 1) lower in unripe inoculated and uninoculated fruits at all storage period of determination although the enzyme activity of uninoculated (2.3 U min⁻¹ g⁻¹ protein) was more or less similar to inoculated (3.0 U min⁻¹ g⁻¹ protein) after 72 h of storage. However, in uninoculated semi-ripe fruits PPO activity was increased about 16.0 fold after 72 h incubation, while in inoculated semi-ripe fruits it was higher (18.0 fold). At the 0 h incubation, the PPO activity was 4.61 fold higher in ripe fruits as compared to semi-ripe fruits but it was increased after 72 h in both inoculated and uninoculated semi-ripe fruits.

These results are consistent with Chakraborty et al., [10] was observed increased polyphenol oxidase in banana fruit infected by *Botryodiplodia theobromae*. Polyphenol oxidase and peroxidase activity gradually decreased during the development of the fruits followed then by an increase during the ripening period. The level of sugar gradually increased during fruit development and ripening which affects the taste of medlar fruits [11]. These observations suggest that the increase in polyphenol oxidase and peroxidase activities as well as in sugar and protein contents has an important role in reducing the astringent taste of banana fruit infected by *Colletotrichum gloeosporioides* [9].

Peroxidase (POX) activity

Peroxidase (POX) activity was found (Table 1) to be higher in uninoculated ripe fruits (48.85-120.9 μmol guaiacol min⁻¹ g⁻¹ protein) than in semi-ripe fruits uninoculated (8.65-60.9 μmol guaiacol min⁻¹ g⁻¹ protein) up to 72 h of storage. The POX activity was enhanced after inoculation with *F. musae* in all ripening stages. The degree of increase in infected unripe, semi-ripe and ripe was 11.71, 7.0, and 2.47 fold higher respectively than uninoculated fruits.

These results are corroborated with earlier findings of Nath et al., [8], the indicated the Peroxidase (POX) activity was found higher in ripe banana fruits than in semi-ripe fruits up to 72 h of storage. The POX activity was enhanced after inoculation with *Lasiodiplodia theobromae* in all ripening stages of banana fruits.

In the present study reduction in PAL activity and enhancement in PPO and POX activity in ripe fruits may be correlated with the reduction of phenol content in ripe fruits. Higher activity of these enzymes in inoculated fruits as compared to uninoculated fruits reflects the invasion of a pathogen. During ripening stages, the level of total phenol gradually decreased in both infected and uninfected fruits, but it was more decreased in infected unripe fruits. The level of total sugar gradually increased during fruit ripening. PAL activity decreased in ripening stages but it increased in infected fruits. PPO and POD activity gradually increased in the ripening stage but the degree of increase was higher in infected fruits.

TSS and total phenol content of banana fruits infected by *Fusarium musae*

Total soluble sugar content (TSS)

Total sugars play a major role in disease resistance since sugars are the precursors for the synthesis of phenolics and phytoalexins which suppress the pectolytic and cellulolytic enzymes that are essential for pathogenesis.

The perusal of data presented in table 2 showed that the total soluble sugar varied in uninoculated and inoculated banana fruits with *F. musae* at unripe, semi-ripe and ripe stages. Total soluble sugar content was higher (83.65mg/g fresh weight) in ripe fruits while lowest (2.10mg/g fresh weight) in unripe fruits at the initial time. Total sugar content was increased at the beginning of the fruit ripening with the increase in the storage period. It was higher (208.65mg/g fresh weight) at 72 h in ripe uninoculated fruits and still further decreases (195.87 mg/g fresh weight) in inoculated fruits and lowest (10.415 mg/g fresh weight) in uninoculated unripe fruits after 72 h of storage. A 9.22 fold increase in total soluble sugar content was observed in unripe inoculated fruits (19.37mg/g fresh weight) followed by unripe uninoculated fruits (10.415mg/g fresh weight) and semi-ripe fruits (173.65mg/g fresh weight) after 72 h of storage. The total

Table 2. Total soluble sugar and total phenol content changes in banana fruits infected by *Fusarium musae*

Total soluble sugar content mg/g fruit weight								
Sr. No.	Sample	0 h.	48 h. after		72 h. after		Fold	
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
1	Unripe	2.101	7.325	12.254	10.415	19.37	+4.98	+9.22
2	Semi-ripe	51.537	85.241	81.27	173.65	167.15	+3.37	+3.24
3	Ripe	83.653	193.35	183.04	208.65	195.87	+2.49	+2.34
Total phenol content mg/g fruit weight								
Sr. No.	Sample	0 h.	48 h. after		72 h. after		Fold	
		Uninoculated	Uninoculated	Inoculated	Uninoculated	Inoculated	Uninoculated	Inoculated
1	Unripe	15.714	13.138	11.61	10.025	8.283	-1.57	-1.90
2	Semi-ripe	13.485	12.379	10.685	10.337	8.732	-1.30	-1.54
3	Ripe	10.592	9.240	8.725	8.984	7.584	-1.18	-1.40

Mean of three replication, *Change over time in uninoculated and **Change over time in uninoculated on inoculated

soluble sugar content was increased with ripening in inoculated as well as uninoculated banana fruits, but it was lower in semi-ripe and ripe inoculated fruits than uninoculated fruits after 48 and 72 h of incubation. These results are corroborated with Terra et al., [12], who found that unripe fruits of banana and mangoes have a relatively higher starch content which is almost completely hydrolyzed during ripening to simple sugars, glucose, fructose, and sucrose [13]. Similar results indicated that increasing of total soluble sugar content in inoculated fruits related to disease development because it is utilized by the pathogen as food for their growth and development [8-9].

Total phenol

Phenolic compounds are the most important group implicated in both constitutive and induced resistance and a distinct correlation between the degree of plant resistance and phenolics present in plant tissue has been demonstrated. Total phenol contents were measured in banana tissue at three maturity stages i.e., unripe, semi-ripe, and ripe fruits in uninoculated and inoculated with *F. musae* after 0, 48 and 72 h of storage. The results presented table 2 that phenol content was higher in unripe fruits (15.714mg/g fruit weight) than semi-ripe (13.485mg/g fruit weight) and ripe fruits (10.592mg/g fruit weight) at the initial time. The phenol content was decreased with increasing storage period i.e., 48 and 72 h in uninoculated and further decreased in inoculated in all three stages. However, total phenol content was decreased as the fruits ripened, and in rotted fruits decrease further with increasing storage period.

Our results are consistent with Nath et al., [8]. The phenol content was decreased with increasing storage period in uninoculated and further decreased in inoculated in unripe, semi-ripe and ripe fruit of banana incited by *L. theobromae* at 0, 48, and 72 h. Similar results were observed by Patel [9] reported that total soluble sugar and total phenol contents was measured in banana tissue at three maturity stages i.e., green mature, semi-ripe and ripe fruits in uninoculated and inoculated with *Colletotrichum gloeosporioides* after 0, 48 and 72 h of storage and observed that total soluble sugar was increased with increasing storage period and phenol content was decreased with increasing storage period in uninoculated and further decreased in inoculated in all three stages.

Conclusion

In biochemical studies, PAL activity was decreased in ripening stages but it increased in infected fruits. PPO and POD activity gradually increased in the ripening stage but the degree of increase was higher in infected fruits. The level of total sugar gradually increased during fruit ripening. The level of total phenol gradually decreased in both infected and uninfected fruits, but it was more decreased in infected green mature fruits.



Abbreviation

PAL- Phenylalanine ammonia lyase, PPO- Polyphenol oxidase, POX- Peroxidase, TSS- Total soluble sugar.

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