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CHANGES IN THE BIOCHEMICAL CONSTITUENTS OF CARROT ROOTS DUE TO BACTERIAL SOFT ROT

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ABSTRACT: Carrot is a rich source of nutrients. The carrot contains carotene and lycopene, which gives bright color to the roots. The quality of the carrots was assessed based on the carotene, lycopene and other biochemical constituents like sugars, starch and protein. To study the effect of various isolates of the *Erwinia carotovora* var. *carotovora* on the above biochemical constituents, the pathogens were inoculated and the contents were analyzed separately at 1, 2, 3, 4, 5 days after inoculation. The contents of β-carotene increased significantly due to all the three isolates of the pathogen and the Coimbatore isolate recorded highest of 36.03 per cent. A same trend was also observed in the lycopene content with 93.55 per cent increase over control. The contents of total and reducing sugars were found to significantly increase due to inoculation with the pathogen. The starch content showed a decreasing trend in all the isolates tested. The maximum reduction of 62.98 per cent was observed in the roots inoculated with Coimbatore isolate.
Key words: Post harvest, Carrot, Soft rot, Erwinia, Biochemical, Diseases.

INTRODUCTION

Carrot *(Daucus carota* L. var. *sativus)* originated from the wild forms growing in Europe and Southwestern Asia (Banga, 1984). The carrots are grown for their prominent root structure and characteristic flavor and colour. It is valued as food mainly because it is rich source of α and β - carotene. In India the carrot is said to have been introduced from Persia. Carrot has been a subject of active research throughout the 20th century. Most of the research has arisen out of practical problems and lacks a sound theoretical background. Moreover, carrot has served as a model plant in plant physiology and more recently in biotechnology.

The bacterial soft rot was found to occur universally and its occurrence has been reported from USA (Farrar *et al*., 2000), New Zealand (Dye, 1953), Poland (Zolobowska and Pospieszney, 2001), India (Ramesh Chand and Ram Kishun, 1989) and Korea (Choi *et al*., 1989). The postharvest loss of carrot at Coimbatore in various markets was 10.61 per cent during the month of October (Abraham, 1999) and he also observed the bacterial soft rot in the samples drawn at different markets.

The quality of the carrots was assessed based on the carotene, lycopene and other biochemical constituents like sugars, starch and protein. To study the effect of various isolates of the soft rot pathogen on the above biochemical constituents, the pathogens were inoculated and the contents were analysed separately at 1, 2, 3, 4, 5 days after inoculation.

MATERIALS AND METHODS

Collection of samples

The infected carrot roots were collected from various markets of Ooty, Coimbatore and Hosur. To assess the bacterial soft rot in the contaminated carrot roots with special reference to *Erwinia carotovora* var. *carotovora*.

Isolation of organism

Carrot roots, showing typical symptoms of soft rot were taken from collected samples and the infected soft portion of roots was surface sterilized with 80 per cent ethanol.Punctures were made on the decayed tissue using sterilized inoculation needle and streaked on the Petri dish containing selective medium (Burr and Schroth, 1977). After 48 h of incubation, the greyish-white growth was purified by the Dilution plate technique (Waksman, 1952). The isolates were maintained in slant by paraffin method (Aneja, 1993).

β- carotene and Lycopene

The contents of β-carotene and lycopene in carrot root were estimated by following the procedure given by Rodriguez *et al.* (1976). Exactly 2.5 g of sample was repeatedly extracted with acetone using pestle and mortar until the residue turned colorless. The acetone extract was transferred to a separating funnel containing 20 ml petroleum ether and mixed gently. Twenty ml of 5 per cent sodium sulphate solution was added and shaken gently. The upper petroleum ether layer was removed and lower aqueous phase was re-extracted with 20 ml more of petroleum ether. The petroleum ether extracts were once again pooled and washed with little distilled water. Ten gram of anhydrous sodium sulphate was added to the petroleum ether and kept aside for 30 minutes. The petroleum ether extract was decanted in to a 100 ml volumetric flask and the volume was made with petroleum ether. The absorbance in spectrophotometer was measured at 435nm for β-carotene and 503 nm for lycopene using petroleum ether as blank. The contents of β-carotene and lycopene were expressed as mg $100gm^{-1}$ of the sample.

Estimation of sugars

One hundred mg of representative root sample was homogenized in five ml of 80 per cent ethanol. The homogenate was centrifuged at 1000 rpm for 10 min and used for the estimation of total sugar and reducing sugar.

Total sugar

Total sugar content was estimated by anthrone method (Hedge and Hofreiter, 1962). Two hundred micro litre of ethanol extract was evaporated using a water bath at 80°C and one ml water was added to dissolve the sugars. Anthrone reagent was prepared by dissolving 200 mg anthrone in 10 ml ice cold 95 per cent sulphuric acid and was prepared fresh before use. Four ml of anthrone reagent was added and the reaction mixture was heated for 8 min. in a boiling water bath and cooled rapidly. The absorbance of the green coloured solution was measured at 630 nm using spectrophotometer. D-glucose was used as standard. The total sugar content was expressed in terms of percentage on fresh weight basis.

Reducing sugar

The method of Somogyi (1952) was followed to estimate the reducing sugar content. Two hundred microlitre of ethanol extract was evaporated using water bath.The residue was dissolved in two ml of distilled water. One ml of alkaline copper tartarate was added and the reaction mixture was heated for ten min. in a boiling water bath and cooled rapidly. One ml of alkaline copper tartarate was added and the volume was made up to ten ml with distilled water (Alkaline copper tartarate was prepared by dissolving 2.5 g anhydrous sodium carbonate, 2.0 g sodium bicarbonate, 2.5 g sodium potassium tartarate and 20 g anhydrous sodium sulphate in 80 ml of water and finally made up to 100 ml).

The reaction mixture was incubated for ten min. at room temperature. The intensity of blue colour was measured at 620 nm using spectrophotometer. D-glucose was used as standard. The reducing sugar content was expressed in terms of percentage on fresh weight basis.

Non-reducing sugar

The difference between total sugar and reducing sugar corresponds to the non-reducing sugar.

Starch

The starch content was estimated by following the method prescribed by Hedge and Hofreiter (1962). One hundred mg of the sample was homogenized in hot 80 per cent ethanol to remove sugars. The residue was retained after centrifugation. The residue was washed with hot 20 per cent ethanol till the washings did not give colour with anthrone reagent.

The residue was dried well in a water bath. To the residue, five ml of water and 6.5 ml of 52 per cent perchloric acid were added. Starch is extracted at 0° C for 20 minutes. The extract was retained after centrifugation. The extraction was repeated with fresh perchloric acid. The extracts were pooled after centrifugation and the volume was made up to 100 ml with 52 per cent perchloric acid. To 0.2 ml of the extract, 0.8 ml of distilled water and 4 ml of anthrone reagent were added. The reaction mixture was heated for 8 min. in a boiling water bath and cooled rapidly. The colour intensity was read at 630 nm using a spectrophotometer. D- glucose was used as a standard and the starch content was expressed as percentage.

Statistical analysis

The data were statistically analyzed using the IRRISTAT version 92 developed by the International Rice Research Institute Biometrics unit, the Philippines (Gomez and Gomez, 1984). Data were subjected to analysis of variance (ANOVA) at two significant levels ($P < 0.05$ and $P < 0.01$) and means were compared by Duncan's Multiple Range Test (DMRT).

RESULTS

Changes in β-carotene

The data from Table 1 indicated that the β-carotene content increased significantly due to the inoculation of all the three isolates. The increase was noticed even from the first day after inoculation. The increase was consistent till the fifth day after inoculation. The maximum increase was noticed in the carrot roots inoculated with the Coimbatore isolate (36.03 per cent), which was followed by Hosur isolate (33.82 per cent).

Table 1. Changes in ß- carotene content of carrot roots due to inoculation of *Erwinia carotovora* var. *carotovora*

Bacterial Isolate	ß- carotene content (mg/100 g)* Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	1.17[b]	1.37[c]	1.54[c]	1.70[c]	1.82[c]	24.19	33.82
Coimbatore strain	1.19[b]	1.47[d]	1.63[d]	1.80[d]	1.85[d]	31.45	36.03
Ooty strain	1.18[b]	1.31[b]	1.46[b]	1.59[b]	1.71[b]	17.74	25.74
Un inoculated control	1.16[a]	1.20[a]	1.24[a]	1.28[a]	1.36[a]		

* Values are the mean of three replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Changes in Lycopene

A progressive increase in the content of lycopene, after inoculation with all the three isolates, was observed from the first day after inoculation with all the three isolates. The increase was noticed till five days after inoculation. The percent increase of lycopene was maximum in the roots inoculated with Coimbatore isolate (93.55 per cent), which was followed by Ooty isolate with 48.39 per cent increase over control (Table 2).

Table 2. Changes in lycopene content of carrot roots due to inoculation of *Erwinia carotovora* var. *carotovora*

Bacterial Isolate	Lycopene content (mg / 100 g)* Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	0.25[a]	0.28[a]	0.36[b]	0.43[b]	0.45[b]	24.14	45.16
Coimbatore strain	0.29[c]	0.37[c]	0.50[c]	0.52[c]	0.60[c]	72.41	93.55
Ooty strain	0.27[b]	0.31[b]	0.37[b]	0.40[b]	0.46[b]	27.59	48.39
Un inoculated control	0.24[a]	0.26[a]	0.29[a]	0.30[a]	0.31[a]		

* Values are the mean of three replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Sugars

Changes in total sugar

The observations revealed that increased reduction in total sugar content was noticed in all the roots inoculated with the bacterium. The reduction was noted even from the first day of inoculation. It was observed that the total sugar content was reduced appreciably in the Coimbatore isolate recording an increase of 28.78 per cent over control, which was followed by Ooty isolate with an increase of 21.30 per cent over control (Table 3).

Table 3. Changes in total sugar content of carrot roots due to inoculation of *Erwinia carotovora* var. *carotovora*

Bacterial Isolate	Total sugar* (per cent) Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rdday	5th day
Hosur strain	6.30 (14.53)[b]	6.30 (14.54)[b]	6.80 (15.11)[b]	7.20 (15.56)[b]	7.49 (15.88)[b]	10.21	19.08
Coimbatore strain	6.58 (14.87)[c]	6.810 (15.12)[d]	7.22 (15.58)[d]	7.97 (16.39)[d]	8.10 (16.53)[d]	17.02	28.78
Ooty strain	6.353 (14.59)[b]	6.61 (14.89)[c]	6.90 (15.23)[c]	7.55 (15.95)[c]	7.63 (16.04)[c]	11.83	21.30
Un inoculated control	5.90 (14.05)[a]	6.10 (14.29)[a]	6.17 (14.38)[a]	6.24 (14.46)[a]	6.29 (14.52)[a]		

* Values are the mean of three replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Figures in parentheses are arc sine transformed values

Changes in reducing sugar

The reducing sugar content showed significant increase in the inoculated roots when compared to control. All the three isolates increased the reducing sugar content. The results are presented in Table 4. The increase was maximum in carrot roots inoculated with Coimbatore isolate recording 27.95 per cent increase over control which was followed by Ooty isolate with 21.21 per cent.

Table 4. Changes in reducing sugar content of carrot roots due to inoculation of *Erwinia carotovora* var. *carotovora*

Bacterial Isolate	Reducing sugar* (per cent) Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	4.90 (12.79)[a]	5.25 (13.18)[b]	5.84 (13.94)[a]	6.50 (14.76)[b]	7.02 (15.36)[b]	10.19	18.18
Coimbatore strain	5.12 (13.07)[c]	5.61 (13.70)[c]	6.20 (14.41)[a]	7.03 (15.37)[c]	7.60 (16.00)[c]	16.98	27.95
Ooty strain	4.80 (12.65)[bc]	5.40 (13.43)[b]	5.76 (13.87)[a]	6.70 (15.00)[a]	7.20 (15.56)[b]	8.68	21.21
Un inoculated control	4.40 (12.10)[ab]	4.90 (12.78)[a]	5.30 (13.31)[a]	5.68 (13.81)[a]	5.94 (14.06)[a]		

* Values are the mean of three replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Figures in parentheses are arc sine transformed values

Changes in non-reducing sugar

The effect of bacterial infection on non-reducing sugar content of carrot roots was studied and the results are presented in Table 5. The results revealed that the non-reducing sugar content of all the carrot roots inoculated decreased from the first day onwards till the fifth day after inoculation. The maximum increase of 42.86 per cent was noticed in the Coimbatore isolate, which was followed by Hosur isolate with 34.29 per cent increase over control.

Table 5. Changes in non-reducing sugar content of carrot roots due to the inoculation of *Erwinia carotovora* var. *carotovora*

Bacterial Isolate	Non-reducing sugar* (per cent) Days after inoculation					Per cent increase over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	1.40 (8.23)	1.05 (5.74)	0.96 (5.62)	0.70 (4.80)	0.47 (3.93)	10.34	34.29
Coimbatore strain	1.46 (7.03)	1.2 (6.29)	1.02 (5.74)	0.94 (5.56)	0.50 (4.05)	17.24	42.86
Ooty strain	1.55 (7.27)	1.21 (6.29)	1.14 (6.02)	0.85 (5.29)	0.43 (3.76)	31.03	22.86
Un inoculated control	1.5 (7.03)	1.2 (6.29)	0.87 (5.35)	0.56 (4.29)	0.35 (3.34)		

* Values are the mean of three replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Figures in parentheses are arc sine transformed values

The above results revealed that the contents of total sugars and reducing sugars increased significantly due to the inoculation of all the three isolates of the pathogen, whereas the non reducing sugar content showed a reverse trend.

Changes in starch

The changes in the starch content of the carrot roots inoculated with pathogen were studied and the results are presented in Table 6. The results revealed that the starch content decreased in the roots inoculated with the pathogen when compared to uninoculated control. The reduction was observed even after one day after inoculation and reached a maximum on the fifth day. On the fifth day after inoculation the reduction in starch content was maximum in the Coimbatore isolate (62.98 per cent), which was followed by Ooty isolate with 61.06 per cent decrease over control.

Table 6. Changes in starch content of carrot roots due to the inoculation of *Erwinia carotovora* var. *carotovora*

Bacterial Isolate	Starch content * (per cent) Days after inoculation					Per cent decrease over control	
	1	2	3	4	5	3rd day	5th day
Hosur strain	2.93 (9.86)^{bc}	2.34 (8.80)^{c}	1.94 (8.01)^{c}	1.23 (6.37)^{c}	0.96 (5.64)^{b}	16.38	53.85
Coimbatore strain	2.61 (9.30)^{a}	2.03 (8.19)^{a}	1.66 (7.41)^{a}	0.89 (5.41)^{a}	0.77 (5.04)^{a}	28.45	62.98
Ooty strain	2.78 (9.60)^{ab}	2.18 (8.49)^{b}	1.85 (7.81)^{b}	0.96 (5.64)^{b}	0.81 (5.18)^{a}	20.26	61.06
Un inoculated control	3.06 (10.08)^{c}	2.74 (9.52)^{d}	2.32 (8.76)^{d}	2.19 (8.51)^{d}	2.08 (8.29)^{c}		

* Values are the mean of three replications

In a column means followed by a common letter are not significantly different from each other at the 5% level by DMRT.

Figures in parentheses are arc sine transformed values

DISCUSSION

β-Carotene and lycopene

Carotenes give carrot its characteristic orange colour. There is a positive correlation between carotene content and colour. Carotene content increased with the age and size of the root (Fritz and Weichmann, 1979; Rosenfeld, 1998). In the present study, the contents of β-carotene increased significantly due to inoculation of all the three isolates of the pathogen and the Coimbatore isolate recorded highest of 36.03 per cent. Same trend was also observed in the lycopene content and 93.55 per cent increase over control was recorded by Coimbatore isolate. Gangopadhyay and Chatopadhyay (1976) correlated carotenoid content with brown spot of rice caused by *Helminthosporium oryzae*. They stated that the carotenoids played an important role in reactions involving oxidation-reduction and protection of sensitive cell enzymes. Carotene content was little affected during storage (Fritz and Weichmann, 1979).

The present study revealed that the contents of β-carotene and lycopene increased significantly due to the inoculation of all the three isolates. The increase was noticed even from the first day after inoculation and was consistent till the fifth day after inoculation. The maximum increase in the β- carotene (36.03 per cent) and lycopene (93.55 per cent) contents were noticed in the carrot roots inoculated with the Coimbatore isolate. These results are in accordance with Prabakar (1999) who reported an increase in β-carotenoid content in mango fruits inoculated with *Colletotrichum gloeosporioides* and Abraham (1999) who reported an increase of 40.56 per cent and 86.13 per cent carotene and lycopene in the carrot roots due to *Fusarium solani* f. sp. *radicicola*. The increase in the contents of β-carotene and lycopene may be correlated with the virulence of pathogens or effective pathogenesis.

Sugars

The contents of total sugar and reducing sugar of the carrot roots increased significantly from the first day of inoculation reaching 28.78 per cent and 27.95 per cent increase respectively in roots inoculated with Coimbatore isolate of the pathogen. The increase in sugars may be due to the conversion of complex carbohydrates into simple forms during pathogenesis by various enzymes. Same trend was also reported by Fladung and Gieffers (1983). They found that infection of *E. caratovora*. subsp. *atroseptica*, *Alternaria alternata* and *Botrytis cinerea* increased the sugar content in potato tubers. Similarly in cassava increase in sugar content occurred due to infection by *Rhizopus oryzae* (Maini and Balagopal, 1978) and *Phytophthora* sp. (Johnson, 1998). Prabakar (1999) reported an increase in total and reducing sugar content in the peel and pulp of mango fruits inoculated with *C. gloeosporioides.*

Abraham (1999) reported an increase in total sugars and non reducing sugar content in the roots inoculated with *F. solani* f. sp. *radicicola.* In the present study also, it was found that the contents of total and reducing sugars showed an increase and this may be correlated with the virulence of pathogens or effective pathogenesis.

Starch

The current investigation indicated that the starch content showed a decreasing trend in all the isolates tested. The maximum reduction of 62.98 per cent was observed in the roots inoculated with Coimbatore isolate. The results are in accordance with Knee (1970) in late blight infected potato tubers, the cassava tubers infected by *Rhizoctonia oryzae* (Maini and Balagopal, 1970), *Phytophthora* sp. (Johnson, 1998) and mango fruits infected by *C. gloeosporioides* (Prabakar, 1999). Abraham (1999) also reported a decrease in the starch content in carrot roots inoculated with *F. solani* f. sp. *radicicola*.

The contents of β-carotene, lycopene, total sugars and reducing sugars were increasing due to the infection of soft rot pathogen, whereas the starch content showed a decreasing trend. These changes may be correlated with the virulence of pathogens or effective pathogenesis.

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