Bangladesh J. Sugarcane, 22 : 71-76 *July, 2000*

Post Infection Changes in Nutrient Composition and Enzyme Activities of Healthy and Red Rot Affected Sugarcane Juice

M. N. Islam, M. H. Rahman, M. F. Hasan[1] and Z. A. Saud
Department of Biochemistry, University of Rajshahi ,
Rajshahi - 6205, Bangladesh.

Received on 09.02.2000

ABSTRACT

The nutrient compositions viz. lipid, protein, total sugar, vitamin-C, calcium and iron of healthy and red rot disease infected sugarcane juice were analyzed. Most of the nutrient contents in sugarcane were affected with infection of fungi. lipid, protein, calcium, iron contents were increased slightly but reducing sugar increased remarkably while only vitamin-C and total sugars were decreased. The activities of amylase, protease and peroxidase increased remarkably but those of invertase, b-glucosidase and catalase increased significantly in diseased infected sugarcane juice as compared to these in healthy sugarcane.

Key words: Red rot, amylase, enzyme, nutrient composition, infectional change, *Colletotrichum falcatum.*

INTRODUCTION

Sugarcane (*Saccharum officinarum* Linn) is one of the most important cash crop in Bangladesh. It is the main sugar producing crop in the country. Sugarcane is vulnerable to various diseases as the crop remains in field for long time and passes through different environmental conditions. Among the various diseases, red rot is the most devastating for sugarcane cultivation. It is reported that the yield and the quality of juices are seriously reduced due to red rot disease (Kamal *et al.* 1981). In case of red rot infection in epidemic from it destroys 80-90 % of mature crop during harvesting season from mid November to March. The millable cane stalks completely dried out and cane only be used as fuel (Ali, 1986). Sandhu *et al.* (1969) reported that sugar content decreases in 1.82 to 31.2% due to red rot infection. Proteolytic and hydrolytic enzymes play some physiological roles during the development of red rot disease in sugarcane caused by *Colletotrichum falcatum.* Therefore the present investigations were carriedout in 1998-99 cropping season to ascertain the changes of essential nutrient contents and enzyme activities in healthy and red rot infected sugarcane.

[1] Scientific Officer (Sugar Chemistry), BSRI, Ishurdi-6620, Pabna

MATERIALS AND METHODS

Apparently healthy and red rot disease infected 10-12 months old sugarcane stalks of variety Isd 18 were collected from the field around Rajshahi sugar mill farm. Sugarcane tissue samples were taken from completely disease free and fully red rot infected internodes of the collected stalks for analysis. Protein content was determined by the method of Micro-Kjeldahl (Jayaraman, 1985). Lipid content of sugarcane juice was determined by the method of Bligh and Dyer (1959). Reducing sugar content was determined by dinitrosalisylic acid method (Miller, 1972).

Total sugar content was determined colorimetrically by the Anthrone method (Jayaraman, 1981). Calcium content was determined by titrimetric method (Bernard and Oser,1965).Iron(Fe) contents of sugarcane juices were determined spectrophotometrically by thiocyanate method (Vogel, 1961). Amount of vitamin-C available in the sugarcane juice was determined by the Roe (1954) titrimetric method. All the reagents used in the experiment were of analytical grade.

Preparation of crude enzyme extract

About 50 gm of sugar cane tissue were cut into small pieces and grinded in a mortar with pestle and then homogenized with cold buffer of respective pH (for amylase and invertase acetic acid-sodium acetate buffer, pH 6, pH 5, respectively and for protease, polyphenol oxidase, catalase, peroxidase with 0.1 M phosphate buffer, pH 7.0, pH 6.0, pH 6.4 and pH 6.0, respectively and for β-glucosidase was 0.1M Na-citrate buffer, pH 4.7). The extracts were filtered with mosline cloth and further clarified by centrifugation at 6000 r.p.m. for 15 minutes and the supernatant was collected and was used as crude enzyme extract.

Amylase activity was assayed following the method as described by Mahadevan and Sridhar (1982). One percent starch solution was used as substrate. One unit of amylase activity is defined as the amount of glucose released from the break down of starch by 1 ml crude enzyme at 15 minutes. Invertase activity was assayed following the method of Mahadevan, and Sridhar (1982). 2.5% sucrose solution was used as substrate. One unit of invertase activity is defined as the amount of glucose and fructose released from the break down of sucrose by 1ml crude enzyme at 15 minutes.

β-glucosidase activities were assayed following the modified method of Lazan *et al.* (1983) using Methyl b-D-glucopyranoside as substrate. Amount of glucose released was estimated by dinitrosalicylic acid method (Miller, 1972). One unit of b- glucosidase activity was defined as the amount of enzyme that catalyzed the liberation of 1 mg of glucose in 15 minutes at 37°C and the amount of glucose released was calculated from the standard curve of glucose. The protease activity was measured according to the method of Kunitz (1947). The milk protein casein was used as a substrate. One unit of protease activity was defined as the amount required for liberating 1 mg of tyrosine per minute at 45°C.

The catalase activity was measured following the method as described by Mahadevan and Sridhar (1982) using H_2O_2 as substrate. One unit of catalase activity is defined as the amount of enzyme which breaks down 1μ mole of H_2O_2 per minute under the assay condition. The polyphenol oxidase and peroxidase activity was measured following the method as described by Mahadevan and Sridhar (1982) using cetechole and pyrogallol as substrate respectively. One unit of polyphenol oxidase activity was defined

as a change in absorbance of 0.001 at 495 nm per min per ml of enzyme extract. In presence of H_2O_2, pyrogallol is oxidized to a coloured derivative-purpurogallin.One unit of peroxidase is defined as the amount of purpurogallin formed per minute under the assay condition.

RESULTS AND DISCUSSION

The nutrient compositions like lipid, protein, vitamin-C, iron, calcium and different enzymes viz. amylase,invertase, β-glucosidase, protease, polyphenol oxidase, peroxidase and catalase contents of healthy and red rot diseased sugarcane juice were analyzed and the results were summarized in Tables 1 and 2. Lipid and protein contents in diseased sugarcane juice increased slightly and reducing sugar increased (81.81%) remarkably Table 1. Increasing the amount of protein is consistent with the increased activity of various enzymes found in infected tissues. Similar results were reported in purple vein virus infected tomato leaves by Nuhu Alam et al. (1960). Singh and waraitch (1977) also reported higher content of protein and nitrogen in infected rose leaves. Total sugar content was found to decrease (30%) after infection with C. *falcatum*. Decrease in total sugar content might be due to conversion to glucose into alcohol and other organic acids as red rot infected cane emit an alcoholic sour smell. Utilization of sugar by the pathogen may be the other causes for reduction of total sugar. Naik et al. (1988) reported that the total sugar content decreased in betelvine leaf infected with *Colletotrichum gloeosporioieds*. Mehta *et al.* (1975) also found lower amount of sugar in fruit-rot diseased tomato. Again, reasonable increase in reducing sugar content at diseased condition might be due to enzymatic conversion of starch and sucrose to reducing sugar. Singh and Waraitch (1977) reported significant increase in reducing sugars in the varieties Co 312, Co 710 Co J 64, Co J 67, Co L 9, Co L 29 and S 909-74 infected with C. *falcatum*. The mean increase in reducing sugar of seven varieties was 203.03%.

Vitamin-C presents in sugarcane juice in a very small amount and the concentration was reduced 35.95% after infection. Reddy et al. (1980) reported that vitamin-C content increase in lemon, musambi and orange fruit infected with *Colletotrichum gloeosporioides* which is contradictory to the present findings.

Calcium and iron contents were found to increase moderately in disease affected sugarcane juices than that of healthy ones. It is probably due to the increased activity of certain enzymes helping in the metabolism of causative organism. Thus it might be concluded that the nutrient compositions are affected greatly after infection with C. *falcatum*.

Table 1. Nutrient compositions of healthy and red rot disease affected sugarcane juice.

Nutrient	Healthy (unit)	Diseased (unit)	Percent increase (+) or decrease (-) over healthy check
Total sugar	10	7	30.00 (-)
Reducing sugar	3.3	6	81.81 (+)
Lipid	0.051	0.06	17.64 (+)
Protein	1.31	1.75	33.58 (+)
Vitamin-C	0.089	0.057	35.95 (-)
Iron	0.04	0.06	50.00 (+)
Calcium	0.015	0.018	20.00 (+)

The activities of different hydrolytic and oxidative enzymes present in healthy and disease affected sugar cane juices were increased sharply after infection with C. *falcatum* (Table 2). The increased activities of carbohydrate splitting enzyme such as amylase, invertase, and β-glucosidase were 300 %, 48 % and 33.34 %, respectively. These might be responsible for the increased amount of reducing sugar in diseased sugarcane juice (Table1). The increased activity of invertase is responsible for decreasing the sucrose content of red rot affected sugarcane juice.

The other hydrolytic enzymes, protease, required by parasite itself for direct cellular penetration increased above five fold in disease affected sugarcane juice than that of healthy one, though the concentration of the enzyme was low in both sources.

Pathogenesis affects a series of biological oxidations in plant tissues (Mahadavan and Sridhar, 1982). Hence the activities of various oxidase enzymes change to a significant extent after pathogenesis. The activities of polyphenol oxidase and peroxidase were found to be changed remarkably while that of catalase was slightly.

The polyphenol oxidase and peroxidase activities in diseased sugarcane juice were about 26.08% and 177.78% higher than that of healthy one. But in case of catalase it increased only by 13.86%. Nema (1991) reported that the activity of polyphenol oxidase and peroxidase were increased by 12-22% and 40-80% in betelvine leaves when it was infected with *Xanthomonas campestris*. Lorrekovich *et al.* (1967) noted that polyphenol oxidase activity increased in *Erwina carolovora* infected potato leaves while Addy and Goodman (1972) observed an increase in peroxidase activity in apple leaves following infection by *Erwina amylovora*.

Table 2. Activity of different enzyme in healthy and red rot disease affected sugarcane juice.

Enzymes	Healthy (unit)	Diseased (unit)	Percent increase (+) or decrease (-) over healthy check
Amylase	11	44	300 (+)
Invertase	25	37	48 (+)
ß- glucosidase	6	8	33.34 (+)
Protease	0.131	0.68	419.08 (+)
Polyphenol oxidase	460	580	26.08 (+)
Peroxidase	36	100	177.78 (+)
Catalase	14	15.8	12.86 (+)

Vir and Grewal (1975) noticed that the activity of catalase increased in gram plant infected by *Ascochyta aabiei*. Tofazzal Hossain *et al.* (1999) found that activities of polyphenol oxidase, peroxidase and catalase were two, five and three times, respectively higher in *Colletotrichum gloesporioides* infected mango leaves than their healthy ones.

REFERENCES

Ali, M.Y. 1986. Sugarcane agriculture and potentiality of cane production in Bangladesh *In* Sugar Industries in Bangladesh M.S. Ahmed, M.H. Hossain and N.M. Sheikh (eds), Bangladesh Sugar & Food industries Corporation, Shilpa Bhaban, Dhaka. Bangladesh. pp. 65-85

Bligh, E.G. and Dyer, W.J. 1959. Total lipid extraction and purification. Can. *Biochem. Physiol.*, **37**: 911-915

Bernard, S. and Oser, L. 1965. Hawk's Physiological Chemistry, 14th ed., McGraw-Hill Book company, New York. pp. 1264-1265

Hayshi, Y. 1962. Amino acid activation in tobacco leaves infected with tobacco mosaic virus. *Virology* **18**: 140-142

Jayaraman, J. 1985. Laboratory Manual in Biochemistry. Wiley Eastern Ltd. New Delhi, India, pp. 73-75

Kamal, Singh, R.P. and Verma, K.P. 1981. Effect of red rot of sugarcane on the yield and juice quality. Co-op. Sug., **12**: 413-414

Kunitz, M. 1947. Crystalline soybean trypsin inhibitor. *J. Gen. Physiol.* **30**: 291-310

Lazan, A.; Ali, Z.M.; Soh., J.S. and Talkah, Z. 1993. Acta Hortic., **341**: 500

Loverkovich, L.; Loverkovich, H. and Stahman, M.A. 1967. Inhibition of phenol oxidation by Erwinia caratovora in potato tuber tissue and its significance in disease resistance. *Phytopathology*, **57**: 737-742

Mahadevan, A. and Sridhar, R. 1982. Hydrolytic enzyme & Oxidative enzyme In Method in Physiological Plant Pathology. Sivakami Publications, Madras. pp. 25-61, 152-159

Mehta, P.K.; Vyas, M. and Saksena, S.B. 1975. Metabolic changes during pathogenesis of fruit rot disease of tomato. *Indian Phytopath.*, **28**: 253-255

Miller, G.L. 1972. Use of Dinitro salicylic acid reagent for determination of glucose. *Anal. Chem.*, **31**: 426-428

Naik, N.K.; Hiremath, P.C. and Hiremath, S.V. 1988. Post infectional changes in the Betelvine Leaves infected with *Colletotrichum gloeosporioides. Indian Phytopath.*, **41**(3): 370-372

Nema, A.G. 1991. Changes in chlorophyll, nitrogen, protein, amino acid and some enzyme contents in Betelvine Leaves infected with *Xanthomnas campestris* Pv. Betlicola. *Indian Phytopath.* **44** (1): 9-14

Nuhu Alam, Abdul Mannan Akanda and Abdul Khair, 1960. Biochemical changes of tomato due to purple vein virus. *Bangladesh J. Bot.*, **25** (1): 25-30

Olah, A.F. and Sherwood, R.T. 1973. Glycosidase activity and flavonotd accumulation in alfalfa infected by Ascochyta imperfecta. *Phytopathology*, **63**: 739-742

Reddy, S.M.; Kumar, B.P. and. Reddy, S.R. 1980. Vitamin-C changes in infected fruits of tomato. *Ind. Phytopath.*, **33**: 511-512

Roe, J.H. 1954. Chemical determination of ascorbic dehydroascorbic and diketoglunic acids. *Meth. Biochem. Anal.*, **1**: 115-139

Sandhu, S.S.; Bhatti, D.S. and Rattan, B. K. 1969. Extent of losses in sugarcane caused by red rot (*Physalospora tucumanensis* Speg.) and (*Ustilago scitaminea* Syd). *J. Research*, **6: 341**-344

Sing, V.K. 1987. Altered metabolism of rose due to yellow mosaic virus. *Bangladesh J. Bot.* **16 (2)**: 215-218

Singh, O.N. and Waraitch, K.S. 1977. Metabolic changes induced by *Colletotrichum falcatum* Went. in sugarcane. *Sugarcane Pathologists' Newsletter*, **27**: 25-30

Tandon, R.N.; Tandon, M.P. and Jamaluddin 1974. Studies on post-harvest diseases of fruits and vegetables. *In* S.P. Raychaudhuri and J.P. Verma (Eds) Current trends in plant pathology. P. 209-220

Tofazzal Hossain, M., Zahangir Alam M. and Absar N. 1999. Changes in different nutrients and enzyme contents in mango leaves infected with *Colletotrichum gloeosporioides*. . *Ind. Phytopath.* **52**(1): 75-76

Vir, S. and Grewal. J.S. 1975. Change in catalase activity of gram plant induced by *Ascochyta aabei infection. Indian Phytopath.*, **28**: 223-225

Vogel, A.I. 1961. A Text Book of Quantitative Inorganic Analysis, 3rd edition. Pub. Longmans. pp. 787, 806