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Determination of Change in Sugar Content in Healthy and Diseased Leaves of Two Mango Varieties (Langra and Chaunsa) affected with Quick Decline Disease

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Abstract: A technically known mango disease called "Collar or stem rot" is now becoming a most destructive one, therefore, nowadays, it is commonly known as "Quick decline". Therefore, the present studywas aimed toestimate the changes in sugar contents of the healthy and diseased leaves of the mango plants of the varieties "Langra" and "Chausa" in relation to "Quick Decline" disease. The obtained results reveals that in general, the metabolism of sugars was found to be disturbed and certain qualitative and quantitative changes appeared to occur in both mango varieties. The sugars detected were lactose, maltose, sucrose, galactose, glucose, fructose + arabinose and xylose. Out of these, sucrose was present in both the leaf tissues of both the varieties. The quantities of total sugars and reducing sugars were found to have decreased in diseased leaves of both the varieties in comparison to healthy ones. To the contrary, the quantities of non-reducing sugars increased in the diseased leaves as compared to healthy ones. Thus, the present study reveals the significance role of sugar contents in the development of defense mechanism in the affected area of leaves against the penetration of "Quick Decline" of mango.

Key words: Mango · Quick decline · Total Sugar content · Reducing Sugar · Non-reducing sugar

INTRODUCTION

Mango is most famous fruit of eastern areas. In all parts of Pakistan it is considered to be most praiseful fruit and oftentimes renowned as the king of fruits [1, 2]. Tropical and subtropical regions contain one of the most famous fruit known as mango(*Mangiferaindica*). It is extensively grown in various countries of the world like Indonesia, Nigeria, Florida, Vitenam, Pakistan, Srilanka, India, Egypt, China, Malaysia, Philippines, Thailand, Qeen land and Java. Pakistan is second biggest country after 1999in production of mangoes in Asia and produces 917,000 tons of mangoes annually [3]. Pakistani mangoes are thought out to be tremendous due to the wonderful taste, delicious flavor and highly nourishing property. They contain large amount of Carbohydrates, proteins, fatty acid, vitamins and amino acids. The mango plants produces the fruits are greatly reduced by various elements and by reason of carelessness of mango grower. Mango undergoes a wide variety of diseases through out of its life. Large number of microbes such as fungai, bacteria and insects are attacked on all parts of plants like leaf, trunks, fruits, flower, branch etc. [4]. A total of 27 mango disease are occurs in mango fruit and mango trees, which are prevailed in Pakistan. Out of 27 mango diseases, quick decline is introduced by *Lasiodiplodiatheobroma* (Pta.), as it is the most severe one and found to be responsible to produce large destruction in Pakistan. The beginning of quick decline of mango becomes apparent by blotch and blackening of small shoot from top end towards the down end. The dark area spread and leaves start dying along the vein at edge and affected leaves turned brown and its margins rolled upward [5]. The most current severe danger to the mango industry of Pakistan is the fungai known as *Ceratocystisfimbriata*, whichis considered to be the first plant microbe related with quick

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decline of mangoesin Oman, Brazil and Pakistan [6]. Though the soil and climatic conditions of Pakistan are suitable for mango production, however, in last few years, the annual production of mango had been found to be significantly decreasing due to some diseases specially "Collar or stem rot" or Quick decline". Therefore, the aim of present research work was to determine the change in sugar content of mango plant leaves of two varieties i.e., Langraand Chaunsa mangoes due to quick decline disease by comparing the healthy and diseased leaves of mango plants. Such information would be valuable to control the intensity of this disease in addition to the pest control and would also be helpful in reducing the risk of enormous loss of mango industry in Pakistan.

MATERIALS AND METHODS

Sample Preparation: the healthy and diseased leaves of mango plants of two varieties i.e., Langra and Chaunsa were collected from the orchard of Shujabad Pakistan. Then leaves were allowed to dry in shade and packed in the plastic bags. Sample was ground into fine powder and stored in the plastic jar [3].

Extraction of Sugars and Amino Acids: One gram of crushed dried leaves in each case were socked in 75% alcohol (40ml) for 24 h and then ground and filtered. The alcoholic extract were filtered into 100ml beakers and placed in an oven at 40-50 °C till the whole liquid evaporated. The residue in each case was dissolved in 75% ethyl-alcohol (3ml) and preserved for the analysis of sugars and amino acids.

Separation of Sugars from Free Amino Aids: The concentrated extract in each case obtained from the 75% alcoholic extract was investigatedfor the contents of sugars and free amino acids. Ion-exchange chromatographic technique was used for the separation of sugars from free amino acids. A column 45cm long with an internal diameter of 1.0 cm was packed with activated action exchange resin (Amberlite IR-120). The resin was poured in the form of slurry, made in the deionized water, with constant stirring for its uniform distribution and to avoid any air trap that can affect the separation. The concentration extract (3ml) was loaded on the top of the resin and column was allowed to remain undisturbed for 5-6 h. The sugars were eluted with deionized water. Five fractions of 100ml each were collected with a flow rate of 250ml/h. A part of each fraction was treated with Molisch reagent (Appendix-II) to test the presence of sugars. Only the first three fractions gave positive test of sugars while the last two fractions showed complete absence of sugars. All fractions were also tested for the presence of amino acids and found to give negative results. The three fractions, which contained all the sugars contents, were mixed together for the estimation of total sugar by anthrone method and reducing sugar by potassium ferricyanide method. The mixed sugar containing fractions (1ml) were used for the estimation of total and reducing sugar. The remaining portion of the mixed fraction was slowly evaporated to dryness in an oven at 40-50°C. The solid material thus obtained was dissolved again in 75% ethyl alcohol (0.5ml) and preserved for the analysis of chromatography.

Qualitative Analysis of Sugar: Whatman No.1 sheets were employed for the paper partition chromatography using ascending techniques for the separation and identification of sugars. The paper sheets (46×57cm) were loaded with the samples coded as S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_8, S_9, S_{10} and the standard sugar. The sheets were dissolved using as solvent system isopropanol: butanol: water (7:1:2). The solvent was allowed to run for 48 h. The developed paper sheets were dried in air and then sprayed with solutions of airline phthalate (Appendix-3). The chromatograms were again dried in air and kept in an oven at 110-120°C for 10-15 minutes. Coloured spots of sugars appeared on the chromatograms. Comparing R_f values with that of standard sugars identified the unknown sugars. The results are given in Table 1.

Determination of Total Sugar Content: Total sugars were estimated by the method of Trevelyanand Harrison[7]. Different volumes of 0.01% Galactose stock solution of 0.1, 0.2,….1.0 ml were taken in separate test tube and volume was made upto 2ml in each case by adding distilled water and then anthrone reagent (4ml) in each test tube was added, after covering the tubes with small glass balls. These were heated in a boiling water bath for 20 minutes and then immediately cooled under running tap water. The blue green color was produced in each case. The absorbance was recorded at 620nm against anthrone reagent prepared in sulphuric acid as reference using UV-Visible spectrophotometer. Galactose standard graph is obtained by plotting the absorbance against known amount of Galactose. The samples (0.1ml) in each case were treated following the same procedure and experimental conditions and absorbance was recorded at 620nm. The total amount of sugars was then calculated in 1g of sample (Crushed leaves) in each case from the standard graph. The results are tabulated in Table 2.

Table 1: Types of sugar concentration in two varieties of mango.

Parameters	Langra healthy	Chaunsa healthy	Langra diseased	Chaunsa diseased
Total sugar	5.13±0.87	3.11±0.012	3.49±0.20	2.88±0.14
Reducing sugar	2.72±0.23	1.90±0.62	1.03±0.17	1.75±0.44
Non-reducing sugar	2.41±0.62	1.2±0.63	2.45±0.047	1.30±0.149

Note: S= amount of sugar, S_1 to S_5 are healthy samples, while S_6 to S_{10} are diseases samples.

Table 2: Total sugar, reducing and non-reducing sugar concentration in two varieties of mango

		Langra healthy		Chaunsa healthy			Langra diseased			Chaunsa diseased	
Name of sugar	R_f value × 100	S_1	S_2	S_3	S_4	S_5	S_6	S_7	S_8	S_9	S_{10}
unidentified	-	96	96	96	96	-	-	-	-	-	-
Xylose	90	90	90	90	90	-	-	-	-	-	-
Arabinose+ Fructose	85	89	89	89	85	87	83	85	-	85	-
Glucose	79	79	81	85	82	-	81	-	81	77	82
Galactose	73	-	-	-	-	73	-	76	75	-	-
Sucrose	65	60	60	60	61	63	65	64	65	60	60
Maltose	47	-	-	48	47	-	46	-	-	-	-
Lactose	29	29	29	29	29	-	29	-	-	-	-

Estimation of Reducing Sugars: Reducing sugars were estimated following the method issued by Hulme and Narain,

- Potassium ferricyanide solution (0.01N) Potassium ferricyanide (1.1g) was dissolved in distilled water and volume was made upto 1.0litre.
- Sodium thiosulphate solution (0.01N) sodium thiosulphate (2.5g) was dissolvedin water and the volume was made upto 1 litre.
- Sodium carbonatesolution (0.1M) sodium carbonate (10.6g) was dissolved in water and the volume was made upto 1 litre.
- Potassium Iodite (5g) zinc sulphate (10g) and sodium chloride (5g) were dissolved in little water and then volume was made upto 200ml.
- Sulphuric acid (3% v/v) concentrated sulphuric acid (3ml) was added in water and volume was made up to 100ml by adding distilled water.
- Starch solution, 1g starch solution was added to the saturated solution of sodium chloride.

To the sample solution (1ml) each taken in conical flasks was added solution of sodium carbonate and potassium ferricyanide (2ml) each. The total volume of reaction mixture was made upto 14ml with addition of water. The reaction mixture was heated on boiling water bath for 15minutes and then cooled. Potassium iodide, zinc sulphate, sodium chloride solution (3ml) was then added to each flask followed by sulphuric acid solution (2ml). Three drops of starch solution were added as an indicator. The reaction mixture was then titrated against sodium thiosulphate solution (Standardized with 0.01N potassium iodide) to a colorless. The total reducing sugars were calculated from the following Hulme and Narain formula. The results of the calculations are shown in Table 2.

S - b (T + a)
S - amount of sugar,
b - a terminal factor (value 0.34)
T - volume of sodium thiosulphate used
a - a terminal factor (value 0.05)
Estimation of non-reducing sugars:
Total sugar – reducing sugar - Non-reducing sugar

RESULTS AND DISCUSSION

The sugar contents of healthy and diseased leaves of the mango plants of the varieties “Langra” and “Chaunsa” were analyzed in relation to the “quick decline”. Generally the metabolism of sugar was found to be disturbed and certain quantitative and qualitative changes appeared to occur. In all eight sugars were detected in the healthy or diseased or leaves of both varieties. Of these, sucrose was present in all the samples regardless of the problem. Similarly, arabinose + fructose were present in both the healthy and diseased leaves of both varieties except those of diseased ones (i.e., samples S_8 and S_{10}). The rest of the sugars viz lactose, maltose, Galactose, glucose and Xylose did not show a uniform pattern of distribution. An unidentified compound was also found to be present in only healthy leaves (S_1- S_4) of both the varieties as shown in Table 1. But not investigated further.

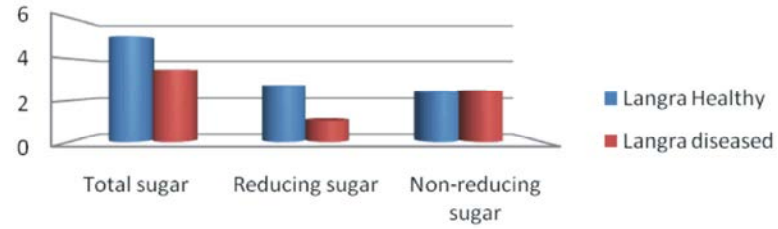

Fig. 1: Sugar content in diseased and health variety Langra

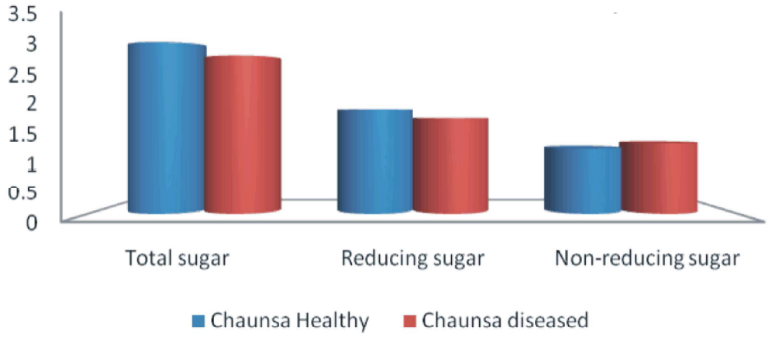

Fig. 2: Sugar content in diseased and health variety Chaunsa

Total sugars of the fruit are considered one of the basic criteria to evaluate the fruit ripening. It is clear from the results that at the time of harvest the sugars were very low but with the passage of time ripening enhances and ultimately total sugars increased [8].The amount of total sugars and reducing sugars were found to have decreased in the diseased leaves of both the varieties as compared to the healthy ones (Figure 1 and 2), which is also noted by Shad *et al.* [3].

In general, reducing sugar was higherat full bloom stage in all the cultivars [9]. The most striking decrease, however, occurred in the case of "Langra". Same result was obtained by the Shad *et al.* [3]. The sugar content decreases in diseased leaves, because the rate of degradation increases and rate of anabolism decreases in diseased leaves because of attacking of microbes. While the reducing sugars also decreases in diseased leaves as compare to the healthy leaves which also has been reported by Marmit and Sharma [4] who also noticed that the reducing sugar is decreased in abnormal tissues of mango leaves. In contrast, the quantities of non-reducing sugars were found to have increased in the diseased leaves of both the varieties (Figure 1, 2), albeit to a smaller extent (Table 1, 2).

CONCLUSION

World is facing challenges regarding the production of mango. The above research is based on the analysis of the sugar content of the diseased and healthy mango of two varieties that are Langra and Chaunsa. An unidentified compound that was also found during the present investigation in only healthy sample leaves (S_1-S_4) of both the mango varieties can be new information that should be investigated further because it can be helpful in determining the cause of health of the mango that are healthy in comparison to those that are diseased one. This research will be helpful in future studies' regarding nutritive value of these two varieties as well as it is also awareness source for the people.

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