# Comparative study on sucrose synthesize gene in some Saudi Arabia date Palm cultivars (*Phoenix dactylifera*)

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**Abstract:** Date palm (*Phoenix dactylifera*) is considered as a food source in many countries as it especially affluent in carbohydrates. Sugars, particularly fructose, glucose and sucrose represent over three-quarters of the dry matter. There are differences in sucrose concentrations between the different date palm cultivars, this may be returned to the expression and activities of the sucrose synthesize gene. So this study aims to estimate the level of expression and activates of this gene and measure the level of different sugars in some cultivars widespread in Saudi Arabia. Leaf samples were collected from different Saudi Arabia date palm cultivars; Rushodia, Barhi, Khadry, Nabtatali, khalas, Ruthana, Wannana, Yellow Sakkari and Red Sakkari from different areas in the Kingdom to estimate the activity and gene expression of sucrose synthase (SS), Sucrose Phosphate Phosphotase (SPP) and Sucrose Phosphate Synthase (SPS). The date showed that sucrose concentrations were significantly deferred between the different cultivars except Barhi, Khadry and Khalas. The highest activities of previous enzymes were observed in Red and yellow sakkari while Khalase and Barhi revealed the lowest activities. Also, based on a semi-quantitative RT-PCR, the highest expressions of these enzymes were observed in Red and yellow sakkari while Khalase and Khadry revealed the lowest activities. Low or high level of sucrose concentration in some cultivars can be illustrated by genetic and environmental factors that may do impact in quantitative and qualitative installation of the sugar fraction by modify the enzymes activity involved in breakdown and processes of synthesis.
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**Key Words:** Date palm (*Phoenix dactylifera*), sugar, enzymes, gene expression.

## 1. Introduction

Date palm (*Phoenix dactylifera*) is a fruit tree with unique nutritional, biochemical and biophysical characteristics, it is a rich source of aesthetic, cultural values, and a genetic resource. It is the only indigenous wild desert plant definitely domesticated in its native harsh environment (Abdullah, 2011). Date fruits have nutritional and value added to features (El Hadrami *et al.,* 2011). They have more than 60% carbohydrates, nearly 10% fat and 5% protein and a large amount of dietary fiber (6-12%). In addition, they considered as a source of several alkali-soluble polysaccharide, oestrone and sterols (El Hadrami and Jameel, 2012).

Dates are especially affluent in carbohydrates. Sugars particularly fructose, glucose and sucrose are formation most of dry matter. The ratio between glucose and fructose varies from 1 to 2 according to ripening stage and cultivar. A small percentage of starch and cellulose are represented in dates (Shinwari, 1993). Elleuch *et al.* (2008) assaying the content of sugar in some date palm cultivars and found that sucrose was dominant in cultivar Deglet Noor, while in cultivar Allig the reducing sugars were more plentiful with an egalitarian percentage of glucose and fructose. They returned this difference to presence a high potential activity of invertase in Allig cv.

Sucrose plays a main role in higher plants metabolism. Which consider as a primary product of carbon fixation, a storage compound, the main sugar transport for long-distance in more plants and acts as a signal molecule (Koch, 2004).

Generally, sucrose is synthesized as one from the end products in green leaves through photosynthesis process. In a special case, its biosynthesis can occur in some developing sink tissues (e.g., tomato and sugar beet root) and non-photosynthetic source tissues (e.g., germinating seeds). In the day, the substrate for sucrose biosynthesis is triose phosphate (TP), which is emitted from the chloroplasts through the TP translocator in exchange for inorganic phosphate (Pi). In the cytoplasm, TP is converted to fructose-1,6-bisphosphate by fructose-1,6-bisphosphate aldolase after that by fructose-1,6-bisphosphatase into fructose-6-phosphate. Fructose-6-phosphate is further modified by phosphoglucoisomerase into glucose-6-phosphate which is then convey into glucose-1-phosphate by phosphoglucomutase. In the following step UTP is joined by UDP-glucose pyrophosphorylase (UGPase) into glucose-1-phosphate to output UDP-glucose with the liberated of inorganic pyrophosphate. Then UDP-glucose (UDPG)

is transformed to sucrose-6-phosphate and sucrose by sucrose phosphate synthase (SPS) and sucrose phosphate phosphatase (SPP), respectively. At the night, from starch mobilization is derived the substrate for sucrose biosynthesis, probably during breakdown of starch by amylolytic. Degradation of the chloroplast starch by phosphorylase produces glucose-1- phosphate which continues to supply triose phosphate for export and for sucrose synthesis (SS). Glucose and maltose produced during amylolysis also reach the cytoplasm, this can occur directly but is mostly via conversion to triose-3-phosphate.

There are many mechanisms for regulate the SS aside from the transcriptional and post-transcriptional regulation of genes encoding for the enzymes of sucrose biosynthesis. The phosphorylation of SPS and its activity decreases that's when increases accumulation of sucrose in the leaves (Siegl *et al.,* 1990). The enzyme has also been shown to function as a substrate for SNF-1 related protein kinases (Sugden *et al.,* 1999) which perhaps important for change its activity. In addition, the activity of SPS is regulated by glucose-6-phosphate and inorganic phosphate which respectively act as activator and inhibitor (Doehlert and Huber, 1983). Lower hexose phosphate exploitation by SS catalyzes the synthesis of fructose-2, 6-bisphosphate, which inhibits the activity of cytosolic fructose-1,6-bisphosphatase (Stitt, 1990). This, as well as lower Pi liberation, leads to carbon detention in the chloroplasts for starch synthesis. The significance of fructose-2,6-bisphophate in controlling SS has been shown in transgenic tobacco where elevated concentrations of this metabolite led to decreased inflows of carbon to soluble sugars, organic acids and amino acids, while promoting the accumulation of starch (Scott *et al.,* 1995).

SS is a key enzyme of sucrose metabolism, catalyzing the reversible conversion of sucrose and UDP to UDP-glucose and fructose. It play a role in supplying energy in companion cells for phloem loading (Fu and Park, 1995), provides substrates for starch synthesis (Zrenner *et al.,* 1995), and supplies UDP-glucose for cell wall synthesis (Haigler *et al.,* 2001). Sucrose phosphate synthase (SPS) is one of a number of sucrose-metabolizing enzymes that regulates the sucrose synthesis pathway. SS can be catalyzed by the coordination of two enzymes in higher plants: SPS (E.C. 2.4.1.14; SPS) and SPP (E.C. 3.1.3.24; SPP) (Huber and Huber, 1996). SPS is known to be a key regulative enzyme responsible for SS in plants (Stitt *et al.,* 1988; Huber and Huber, 1992; Huber and Huber, 1996). All sucrose-synthesizing organisms contain SPS, which is regulated by several interacting mechanisms, including: (i) Regulation of gene expression (Huber and Huber, 1996), (ii) Covalent modification via reversible phosphorylation (Huber and Huber, 1996), and (iii) Allosteric regulation via metabolites (Doehlert and Huber, 1985; Mu-Ho *et al.,* 2005).

There are differences in sucrose concentrations between the different date palm cultivars, this may be returned to the expression and activities of the sucrose synthesizing enzymes. So it can be used as tolls for study the biodiversity in the different palm date cultivars in KSA.

So this study aims to estimate the level of expression and activates of these genes and measure the level of different sugars in some cultivars widespread in Saudi Arabia.

## 2. Material and methods

Leaf samples were collected from different Saudi Arabia date Palm cultivars (*Phoenix dactylifera*) from different areas in the Kingdome and kept in liquid nitrogen until be used for estimation of sucrose synthetase, Sucrose Phosphate Synthase, Sucrose Phosphate Phosphatase genes expression, the other part were kept under frizzing till be used for determination of sucrose synthetase enzyme, Sucrose Phosphate Phosphatase, Sucrose Phosphate Synthase activities .At the same time fruits from the same cultivars were collected and prepared for assays of different carbohydrates.

### 2.1. Carbohydrate monitoring:

#### 2.1.1 Extraction of carbohydrates

Carbohydrates were determined in the sample leaves and fruits by the method of Caporn et al. (1999). Firstly the soluble carbohydrates were extracted from both fruits and leaves in ethanol and incubation at 70 °C. Then the extract stored at -80 °C till be used.

#### 2.1.2 Separation of Soluble carbohydrate

The extract was desiccated in a vacuum oven at 40 °C for overnight then the remainder resolublized in distilled water and stockpiled at -20 °C.

#### 2.1.3 Enzymatic determination of carbohydrates:

Glucose, fructose and sucrose were determined using plate reader. Plates load with 50 µl of extract, 160 µl of HEPES buffer, 10 µl NAD, 10 µl ATP and 0.5 units of Glc6P dehydrogenase and either hexokinase (0.5 units) for glucose, or phosphoglucoseisomerase (0.6 units) for fructose, and invertase (8 units) for sucrose then determine the absorbance difference at 340 nm.

### 2.2. Biochemical determination of sucrose metabolizing enzymes activities:

#### 2.2.1 Determination of sucrose synthase activity (SS).

Sucrose synthase was assay through monitoring of the rate of sucrose synthesis through incubation of the leaf extract in HEPES in presence of UDPG,

fructose, and MgCl2. Then the reaction stopped after different time intervals by boiling in presence of KOH for 10 min, and Sucrose was assayed following van Handel (1968), and the rates of sucrose synthesis calculated by the method of Echeverria, 1992.

**2.2.2 Determination of sucrose phosphate synthase activity (SPS).**

The SPS activity was monitored in reaction mixture containing 100 mM HEPES 7.5 mM UDPG, 18 mM MgCl2, 7.5 mM fructose- 6-phosphate, 1.0 mM EDTA, 37.5 mM glucose-6-phosphate and 100 µL of leaf extract and its activity calculated as sucrose synthase.

**2.2.3 Determination of sucrose phosphate phosphatase activity (SPP).**

The activity of SPP was monitored through monitoring of the liberated of inorganic phosphate when the leaf extracted incubated in a mixture of MES, MgCl2,S-6-P, and the reaction stopped at different times intervals by mixing 50 µL of the reaction mixture with 250 µl of 7.2% SDS then the liberated inorganic phosphate was determine at 850 nm according to Chifflett *et al.* (1988).

**2.3. Molecular assays and gene expressions:**

Determination of sucrose synthetase, Sucrose Phosphate Synthase, Sucrose Phosphate Phosphatase gene expression using a semi-quantitative RT-PCR according to Meadus (2003).

Total RNA preparation, RT-PCR and PCR was done using the suitable kits following the manufacture instructions and using the suitable primers.

**2.4 Statistical analysis**

The acquired data were analyzed and graphically represented using the SPSS 18.0 software 2011, for obtaining standard error and means. Duncan's test was used for making a multiple comparisons among the groups for testing the inter-grouping homogeneity.

## 3. Results

**3.1. Sucrose, glucose and fructose concentrations in the different date Palm cultivars.**

Sucrose concentrations were significantly defer between the different cultivars except Barhi, Khadry and Khalas. The highest concentration were observed in Red sakkari then Yellow sakkari while the lowest in Khalas. Controversy to the sucrose concentrations the highest glucose and fructose concentrations were present in Barhi and Khalase and the lowest in Nabtatali and Sakkari (Table 1)

**Table 1.The concentration of sucrose, glucose and fructose (g/100g) in deferent dates cultivars**

| | Rushodia | Barhi | Khadry | Nabtatali | khalas | Ruthana | Wannana | y. Sakkari | R. Sakkari |
|---|---|---|---|---|---|---|---|---|---|
| **Sucrose** | $15.8 \pm 0.8^{d}$ | $1.9 \pm 0.1^{f}$ | $2.7 \pm 0.6^{f}$ | $31 \pm 1^{c}$ | $1.5 \pm 0.5^{f}$ | $13.3 \pm 0.4^{d}$ | $9.3 \pm 0.9^{e}$ | $38 \pm 2^{b}$ | $51 \pm 2^{a}$ |
| **Glucose** | $22 \pm 1^{d}$ | $56.3 \pm 1.5^{a}$ | $28 \pm 2^{c}$ | $8.6 \pm 0.4^{f}$ | $31.6 \pm 1.5^{b}$ | $28.3 \pm 3^{c}$ | $26 \pm 3.6^{c}$ | $13 \pm 1^{e}$ | $12.8 \pm 0.76^{e}$ |
| **Fructose** | $21.3 \pm 1.5^{c}$ | $44.7 \pm 3.7^{a}$ | $25.3 \pm 2.5^{bc}$ | $7.8 \pm 0.2^{e}$ | $45 \pm 5^{a}$ | $23.7 \pm 4^{bc}$ | $27 \pm 3.6^{b}$ | $15.7 \pm 0.6^{d}$ | $10.1 \pm 0.17^{e}$ |

The values refers to means ± SD

Deferent symbols assigned to difference significant between cultivars at $p \leq 0.05$.

**3.2 SS, SPS and SPP activities in the different date Palm cultivars.**

The activities of SS, SPS and SPP showed a different pattern in the studded date Palm cultivars, the highest activities were observed in Red and Yellow sakkari while Khalase and Barhi revealed the lowest activities (Table 2).

**Table 2. The activities of SS, SPS and SPP in the deferent date palm cultivars.**

| Parameters µmol·min$^{-1}$·g$^{-1}$ fresh weight | Rushodia | Barhi | Khadry | Nabtatali | khalas | Ruthana | Wannana | y. Sakkari | R. Sakkari |
|---|---|---|---|---|---|---|---|---|---|
| **Sucrose Synthase** | $14 \pm 1^{d}$ | $7.2 \pm 0.3^{g}$ | $8.4 \pm 0.2^{fg}$ | $25.3 \pm 2.5^{c}$ | $6.2 \pm 0.3^{g}$ | $10.8 \pm 0.8^{e}$ | $10 \pm 1^{fe}$ | $28 \pm 1^{b}$ | $32.3^{a}$ |
| **Sucrose Phosphate Synthase** | $12 \pm 1^{c}$ | $4.9 \pm 0.1^{de}$ | $5.6 \pm 0.05^{de}$ | $22 \pm 3.6^{b}$ | $3.3 \pm 0.2^{e}$ | $11 \pm 1^{c}$ | $8.5 \pm 0.5^{cd}$ | $25.6 \pm 4.9^{b}$ | $32.3 \pm 2.5^{a}$ |
| **Sucrose Phosphate Phosphatase** | $11.3 \pm 1.5^{d}$ | $4.2 \pm 0.2^{ef}$ | $5.2 \pm 0.2^{ef}$ | $42.7 \pm 2.5^{c}$ | $2.8 \pm 0.5^{f}$ | $11.7 \pm 1.5^{d}$ | $8.9 \pm 1^{de}$ | $52.6 \pm 8.7^{b}$ | $62.6 \pm 2.5^{a}$ |

The values refers to means ± SD

Deferent symbols assigned to difference significant between cultivars at $p \leq 0.05$.

**3.3 SS, SPS and SPP genes expression in the different date Palm cultivars.**

The gene expression of SS, SPS and SPP showed a different pattern in the studded date Palm cultivars, the highest expressions were observed in Red and yellow sakkari while Khalase and Khadry revealed the lowest activities (Table 3 and Figure 1).

**Table 3: SS, SPS and SPP genes expression (relative expression to 18srRNA) different date Palm cultivars.**

| | Rushodia | Barhi | Khadry | Nabtatali | khalas | Ruthana | Wannana | y. Sakkari | R. Sakkari |
|---|---|---|---|---|---|---|---|---|---|
| **Sucrose Synthase** | 12.3 ± 2 [c] | 3.3± 0.6 [e] | 0.8± 0.2 [f] | 32.6± 6.4 [b] | 0.3± 0.02 [f] | 10± 1 [c] | 7± 1 [d] | 49.3 ± 4 [a] | 51 ± 2.6 [a] |
| **Sucrose Phosphate Synthase** | 10 ± 1.6 [c] | 3.1 ± 0.7 [e] | 0.8 ± 0.05 [f] | 27 ± 5 [b] | 0.27 ± 0.001 [f] | 8 ± 0.9 [c] | 5± 0.4 [d] | 32.5± 3 [a] | 31 ± 5 [a] |
| **Sucrose Phosphate Phosphatase** | 9± 1.3 [c] | 3 ± 0.6 [e] | 0.6 ± 0.02 [f] | 21.7± 3 [a] | 0.25 ± 0.0023 [f] | 6.5 ± 0.7 [c] | 4,6 ± 0.9 [d] | 25.6 ± 4 [a] | 27.4 ± 6 [a] |

The values refers to means ± SD

Deferent symbols assigned to difference significant between cultivars at $p \leq 0.05$.

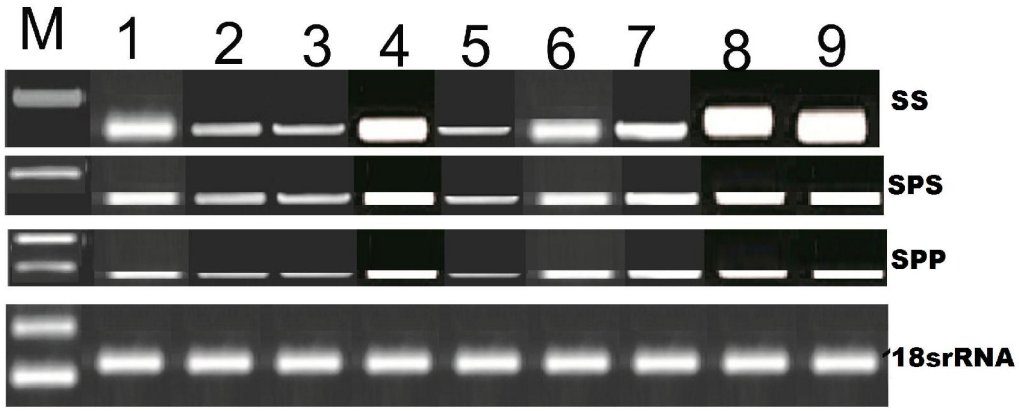


**Fig. 1: The expression level of mRNA of SS, SPS and SPP genes relative expression to 18srRNA. M: Marker; 1: Rushodia; 2: Barhi; 3: Khadry; 4: Nabtatali; 5: khalas; 6: Ruthana; 7: Wannana; 8: y. Sakkari; 9: R. Sakkari.**

### 3.4 Correlations between SS, SPS and SPP activities, Sucrose, glucose and fructose concentrations.

There were a positive correlation between SS, SPS, SPP activities and sucrose concentrations, while there are a negative correlation between them and both glucose and fructose concentrations. At the same time there were a negative correlations between sucrose and both glucose and fructose concentrations, (Table 4).

**Table 4: Correlations between SS, SPS and SPP actvties, Sucrose, glucose and fructose concentrations.**

| | SYS | SPS | SPP | SU | GLU | FRU |
|---|---|---|---|---|---|---|
| **SYS** | 1 | .973** | .985** | .975** | -.761** | -.812** |
| **SPS** | .973** | 1 | .955** | .969** | -.734** | -.808** |
| **SPP** | .985** | .955** | 1 | .975** | -.723** | -.767** |
| **SU** | .975** | .969** | .975** | 1 | -.753** | -.808** |
| **GLU** | -.761** | -.734** | -.723** | -.753-** | 1 | .869** |
| **FRU** | -.812** | -.808** | -.767** | -.808** | .869** | 1 |

**. Correlation is significant at the 0.01 level (1-tailed). N=27

## 4. Discussion

Dates sugars are consist of a mixture of sucrose, glucose and fructose (Barreveld, 1993; Ali and Aldosari, 2007). The fruit of date are high-energy food sources with 72 – 88 % sugar content at maturity (Rastegar *et al.,* 2012). Sugars in date flesh mainly consist of fructose, glucose and sucrose. They are found as predominant sugars in dates from different cultivars at maturation, but with significant differences in proportions between the cultivars. The majority of date cultivars are characterized by a high quantity of reducing sugars (glucose and fructose) and low or zero amount of sucrose. The rapid build-up of fructose and glucose from khalal onwards distinctly indicates that the date is an excellent source of readily available carbohydrates (Rastegar *et al.,* 2012).The sugar levels are known to change during the development of the fruit and the process generalized as maturation and ripening (Eltayeb *et al.,* 1999). Through the Khalal stage, almost all (80 – 85 %) of

the sugar is sucrose, as ripening progresses, it is hydrolyzed to reducing sugars like fructose and glucose (Chao and Krueger, 2007). Sawaya *et al.* (1983) classified dates into two type, dates containing sucrose and dates containing reducing sugars. In general Saudi dates contained high levels of the non-reducing sugar sucrose (Eltayeb *et al.,* 1999).

The present results define three group of dates first with high concentration of sucrose and low concentration of reducing sugars as Nabtatali, Y. Sakkari and R. Sakkari (Table 1) .El-Ghazali and Hussein (2003) studied the effect of sun-drying and mechanical drying on, chemical composition, lipid and phospholipids fractions of Aswan dry-dates, they reported that non-reducing sugar was higher in Bartamuda and Goundaila than Sakkoti (Tamar) and arranged between 58- 64 % while reducing sugar was higher in Sakkoti than Bartamuda and Gondaila and arranged between 16- 23 %. Abd-Almagad and Elrdimman (2003) studied sugar content of date varieties produced in Al-Qassim region and its relationship to the salinity level of irrigation water and soil was investigated, the amounts of reducing sugars was 11.2 - 67% and sucrose 0 -28.4%. The high activity and gene expression of sucrose synthesizing enzymes in these date cultivars ( Tables 2 and 3 and Figure 1) as well the positive correlation between sucrose synthesizing enzymes activities and sucrose content may be a potent explanation to the high sucrose content sucrose in these date cultivars. At the same time the negative correlation between the activities of sucrose synthesizing enzymes and the reducing sugars (glucose and fructose) (Table 4) may be good explanation to the low content of these sugars in these cultivars. (Anne Whittaker *et al.,* 2007) reported that in the initial phase of dehydration, photosynthesis and starch content declined to immeasurable levels, whilst significant increases in hexose sugars, sucrose, and amino acids were associated with concomitant significant increases in SPS and pyruvate kinase activities, and maximal activity levels of phosphoenolpyruvate carboxylase, NADP dependent isocitrate dehydrogenase, and NADH-dependent glutamate synthase. The highest levels of SPS activity corresponded to the maximum rate of net sucrose accumulation. Their results suggesting that sucrose accumulation may compete more strongly for carbon entering glycolysis during the period of hexose phosphorylation were provided by the maximal levels of SPS activity and protein together with the potential of the enzyme to withstand inhibitory conditions coinciding with increased rates of sucrose accumulation. The hexose phosphate sugars do not accumulate during hexose sugar phosphorylation (Whittaker *et al.,* 2001), and that SPS activity is maximal, indicates a utilization of carbon by SPS to support the increased rates of net sucrose accumulation. Levels of sucrose accumulated in their investigation were comparable with those previously reported for S. stapfianus (Whittaker *et al.,* 2001), and hexokinase activity similarly paralleled SPS activity, in support of previous observations that sucrose accumulation occurs concomitantly with increased synthetic activity (Whittaker *et al.,* 2001). A positive correlation between sucrose synthase activity, sink strength and sucrose import has been demonstrated in many sink organs, suggesting a role for this enzyme in carbohydrate partitioning between sink and source organs (Sung *et al.,* 1989; Wang *et al.,* 1993; Zrenner *et al.,* 1995).

Second group with very low concentration of sucrose and high concentration of reducing sugars as Khalas, Barhi and Khadry (Table 1). Sawaya (1986) and Sawaya *et al.* (1983) studied the sugar, tannins and some vitamins content of twenty-five date cultivars grown in Saudi Arabia at the Khalal and Tamer stages. The total sugars and reducing sugars were, in general, higher in the Tamer stage while sucrose was higher in the Khalal stage. In all the cultivars, glucose and fructose were the only detected monomers. It was concluded that in general, the amounts reducing sugars 29 -85% and sucrose 0-43%, at Tamer stages. Rastegar *et al.* (2012) studied the amount and type of sugar change according to variety and maturation stage in Iranian date palm. Reducing sugar changed from 23 to 63, 12.9 to 52.6 and 12.4 to 54.3 in Shahani, Piarom and Deiry respectively, through fruit growth with Shahani showed significantly higher content of reducing sugar compared to other varieties. These results are, in general, comparable with those published previously on different date varieties (Al-Hooti and Qabazard, 1997). Low Sucrose was detected in Shahani and Piarom but Deiry has higher sucrose content. Results obtained for the Shahani are in agreement with those reported by Maghsoudlou *et al.* (2005) (1.7 g/100 g sucrose) (Rastegar *et al.,* 2012). Low activities and gene expression of sucrose synthesizing enzymes in these date cultivars (Table 2 and 3 and Figure 1) as well the positive correlation between sucrose synthesizing enzymes activities and sucrose content may be a potent explanation to the low sucrose content sucrose in these date cultivars. At the same time the negative correlation between the activities of sucrose synthesizing enzymes and the reducing sugars (glucose and fructose) (Table 4) may be good explanation to the high content of these sugars in these cultivar. Ahmed and Robinson (1995) found that the sugar content in 12 different varieties of dates grown in the United Arab Emirates varied from 44.3 to 64.1 g/100 g in tamar stage. Low Sucrose was detected in some varieties can be explained by the

environmental and genetic factors that may affect the qualitative and quantitative composition of the sugar fraction by altering the activity of the enzymes involved in synthesis and breakdown processes. In general, sucrose underwent a complete hydrolysis into reducing sugar at tamar stage. The content of reducing sugars depends on cultivar and is closely related to texture (Rastegar *et al.,* 2012). The soft dates with high humidity are very low amounts on sucrose. For semi-soft dates (Aziza Bouzid and Deglet Nour) sucrose accumulated at the end of maturity which made them palatable (Ahmed & Robinson, 1995).

Third group with medium concentration of sucrose and high concentration of reducing sugars as Wannana, Ruthana and Rushodia ( Table 1), at the same time have a medium actvties and gene expression of sucrose synthesizing enzymes ( Tables 2,3 and Figure 1).

It cane concluded that, the studded date cultivars are divided into 3 groups depending on its reducing and non reducing sugars content. The sugar content either reducing or non reducing in date cultivars are correlated to the activities and gene expression of the sucrose synthesizing enzymes.

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