Mechanisms of date palm *Phoenix dactylifera* salt tolerance, effect of water salinity on some cell wall enzymes activity during development of Sayer date palm fruit

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Abstract. The activities of polygalacturonase (PG), pectinesterase (PE), cellulase (Cx) and polyphenol oxidize were investigated in Sayer date palm fruit cultivar were arrogation by two different water salinity (16 dsm and 4 dsm) during fruit development. The date palm *Phoenix dactylifera* fruit has single sigmoid growth curve with three stages (the first stage was 5 weeks and the second stage was 12 weeks whereas the third stage was 4 weeks). The pectin content was high during early stage of fruit development, but declined gradually with fruit development and reached their lowest value when the fruit entered the ripening phase. The PG, PE and Cx were low level during early stages of fruit development, but there were rapid increase in these enzymes activity as the date palm entered variation stage and maturity phase, the water salinity was a significant effect in all ripening enzymes.
Key Words: salt tolerance, polygalacturonase (PG), pectinesterase (PE), cellulase (Cx).

Introduction. Salinity is a most famous problem in an arid or semi-arid region because in the last years the soil and water salinity become high. The salinity effect plants as scientists define by two major ways, osmotic or water-deficit and salt-specific or ion excess, in general, the salinity affect all chemical and physiological process of the plant as well as water relationships, growth, photosynthesis, ions uptake. Date palm *Phoenix dactylifera* is one of the few fruit tree which is tolerant to water and soil salinity, the mechanism of this tolerance is not yet known. There are several hypotheses about the *P. dactylifera* salinity bearing such as the ability toward mineral selection from soil by roots and there are many osmoprotective compounds in leaf which can help it to tolerate and therefore the leaves structures help in resistance. Many researches has been reported in this field (Abdulwahid 2012a,b; Abdulwahid et al 2016) but all this papers take as subject the plants in offshoot stage not on adult stage, because the salinity study on adult plant is difficult due to plant adaptation and root distribution. But in this matter, the nature helped us when the salinity in the Shat Alarab river (one of the biggest rivers in Iraq region) become more saline in last year's, therefore the treatment in the present paper was already applied. In this paper we are investigating the effect of water salinity on the most important characteristics, on ripening parameters (ripening enzymes) and ripening relation with salinity.

Ripening is the physiological and chemical process in fruits that causes them to become more palatable. In general, a fruit becomes sweeter, less green, and softer as it ripens (Abdulwahid 1998; Abdulwahid 2011).

Fruit ripening is a genetically programmed process of high precision, in which many hormones and enzymes are active within the fruit until they reach full ripening, but they are influenced by many environmental factors such as salinity. Cell walls play a key role in cell protection and in the regulation of intercellular exchanges then play important changes in fruit ripping (Abdulwahid 2011).

Enzymes which play a key role in chemical ripening changes is cell wall degradation enzymes and sugar inversion enzymes are: invertase (Inv), pectinesterase

(PE), polygalacturonase (PG), cellulose (Cx) and many other enzymes (Abdulwahid 2011).

PE is an enzyme widely found in plants and microorganisms, which hydrolyzes the methyl ester bods of the esterified carboxyls, releases methanol and transforms the pectin in low methoxyl pectin and even polygalacturonic acid. PE has been extracted and characterized as part of the ripening behavior of kiwi (Wegrzyn & Macrae 1992) pear (Zhou 2000), and guava (Abu-Bark et al 2003). It has been proposed that the control of the enzymatic action will control the process of softening, which will result in an increase in the commercialization of the fruits and a better profit and ratability of the fruits.

During fruit ripening, PG is mainly responsible for the dissolution of the middle lamella. Exo PG breaks down pectin by hydrolysing the α- 1,4-glycosidic bonds between the galacturonic acid residues in galacturonans from the non-reducing end, which results in the release of galacturonic acid as the major reaction product (El-Batal et al 2013). Also during the ripening of some fruits, cellulases are produced to break down the cellulose in cell walls causing softening. All these enzymes contribute to the increased of the softness of the fruit and then repining, the changes in these enzymes during growth and repining of fruit have been studied by many researchers, in tomato (Ali & Abu-Goukh 2005), pear (Liu et al 2008), date palm (Abdulwahid 2011). Because there were a few studies on the cell wall enzymes of jujube fruit, and it played an important role in fruit ripening, therefore we are focused in this paper to investigate changes in pectin substances and the activity of the cell wall degrading enzymes PE, PG and Cx during jujube fruit ripening.

Material and Method. The present study was conducted during the fruit growing season in 2014, private orchard at AL_Shat Alarab region near the river, in this year the salinity water increased to 16 dsm. We have selected two water salinity water treatments, river water (16 dsm) and diluted river water of four-time (4 dsm) by distill water. The *P. dactylifera* tree was 10 years old and the tree was irrigated twice in a week till soil saturation.

Sampling collection and preparing. The samples were collected from 6 plants of each treatment which were planted with a row spacing of 7 × 7 m, all of the plants were grown in accordance with local management standards, including pruning, fertilizing and pest control. Samples were collected once every 15 days after the fruit set stage until the fruits ripened. The fruits were considered mature when the skin color changed from green to light yellow (Kallal stage) whereas the ripening stage when fruit take brown at the fruit tip and the skin be more soften (Rotab stage). Five fruits were collected at each sampling. The samples were randomly collected from trees and the fleshy tissue of the 5 fruits was cut into small pieces and mixed together. Approximately 10 g of each sample was placed in a freezing vial and stored at -5°C until use (Abdulwahid 2011).

Fruit physiological characters. Samples of 60 fruits were taken randomly from each tree (each 2 trees is replicate) to determine fruit weight.

Pectin content. Pectin was determined according to (Pearson 1970).

Enzymes assay. Extraction and estimation of plant enzyme (PE, PG, Cx, PPO) was performed according to (Abdulwahid 2011).

Statistical analysis. The statistical analysis of the data was performed using the general linear model (GLM) and analysis of variance (ANOVA) technique. The means were separated by least significant difference (LSD). The statistical analysis was performed with the statistical software package (SPSS v.19).

Results and Discussion. The growth curve of Sayer cv. is shown in Figure 1. We were noted that the growth curve was single sigmoidal with three growth phase, stage I was characterized by slowly increased in fruit weight, this period requires approximately 5

weeks. Then the fruits were entered into the lag phase of growth. The weight of fruit resumed rapid growth rate and this stage lasted for 7 and 9 weeks in 4 dsm and 16 dsm respectively, at the end of this stage the fruit was entered in maturity then ripening, we were noted the high level of water salinity increased the long period of stage II, and the fruit was ripening late, in addition, the fruit weight in water salinity was more decreased at 16 dsm, compared to 4 dsm.

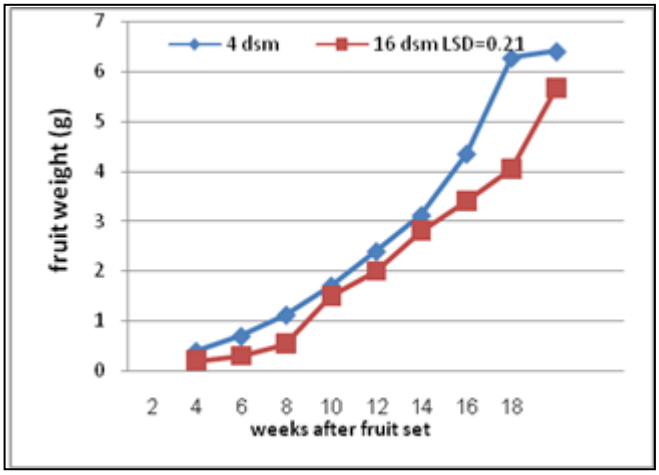

Figure 1. Effect of water salinity on fruit weight during fruit growth and development in *Phoenix dactylifera*.

Pectin content. Figure 2 showed changes in pectin content during the fruit development of the two treatments (4 and 16 dsm). Maximum pectin content was recorded in 6 weeks after fruit set of 2.9% and 2.6% of in 4, 16 dsm respectively. Pectin content (Figure 2) also showed a continuous decline from the first stage to end of the second stage. Then the pectin was rather stable at end of the second stage between 10 and 12 weeks, then the pectin content resumed rapid decline till the fruit ripening. The water salinity effect was significant, the fruit irrigated with 4 dsm water recorded more pectin then fruit irrigated with 16 dsm.

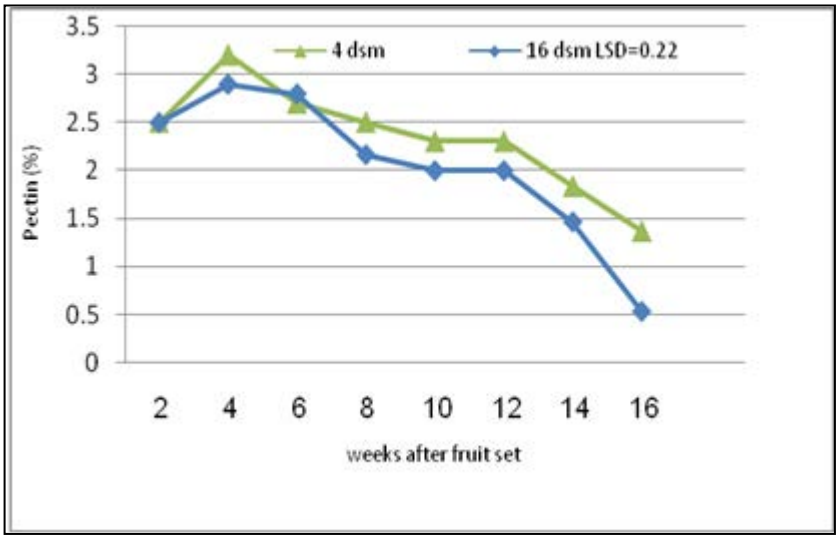

Figure 2. Effect of water salinity on pectin content during fruit growth and development of *Phoenix dactylifera* Sayer cultivar.

Changes in enzymes activity. The PG activation slightly increased untill 8 and 10 weeks after fruit set, in both treatment 4 and 16 dsm respectively, then sharply increased, reaching the peak at 12 and 14 weeks after fruit set, which coincided with the variation of fruit color (Figure 3).

Figure 4 shows changes in PE enzyme activity (microequivalent/kg/min) during fruit development of two treatments (4 dsm and 16 dsm). The activity of PE was absent and not detectable at first the stage of fruit development then the PE activity was a little low and increased along with fruit development up to the end of stage II. It is obvious that the activity was low during the first 8 weeks of fruit development from fruit set, but there was a rapid increase in this activation when entered venison. Then the PE activation declined till the end of fruit ripening.

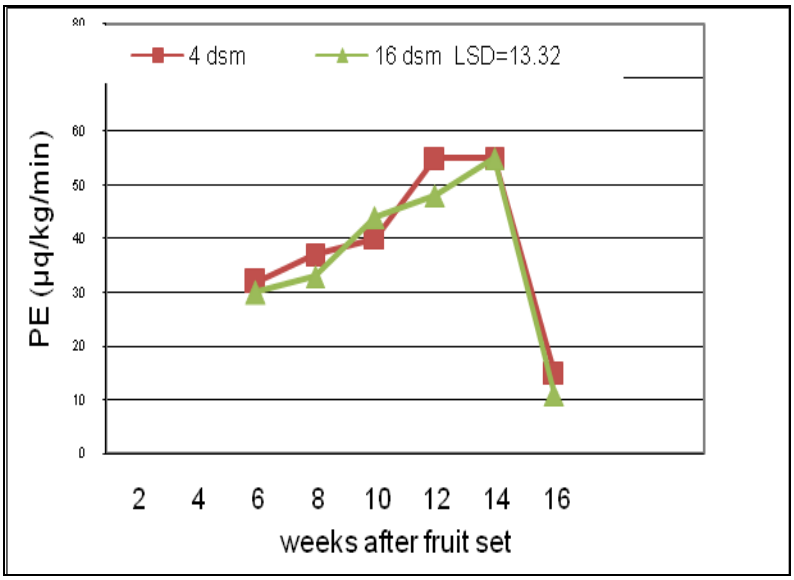

Figure 3. Changes of pectinesterase (PE) activity during fruit growth and development of *Phoenix dactylifera* Sayer cultivar.

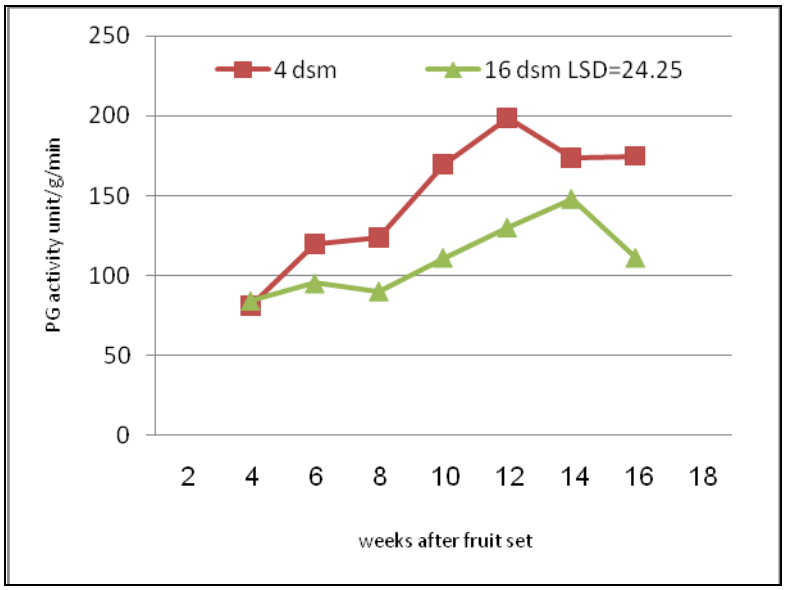

Figure 4. Effect of water salinity on polygalacturonase (PG) activity during fruit growth and development of *Phoenix dactylifera* Sayer cultivar.

Cellulase activity increased gradually along with fruit development. It rises dramatically from 2 to 4 weeks after fruit set and from 12 to 14 weeks after fruit set for 4, 16 dsm

respectively (Figure 5). The effect of water salinity on enzymes activity has no significant effect.

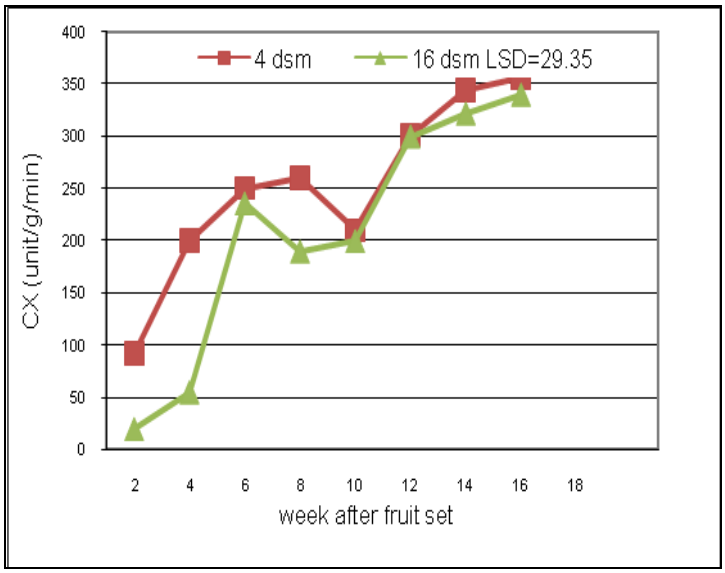

Figure 5. Effect of water salinity on cellulase (Cx) activity during fruit growth and development of *Phoenix dactylifera* Sayer cultivar.

Discussion. The first growth stage may be caused by cell division and cell enlargement. Abdulwahid (2011) and Abbass & Fandi (2001a, b) found that there was a higher level of promoter hormones (IAA, GA3, and CY) in the first stage of fruit, therefore, this stage is active in cell division and cell enlargement. Then when fruit growth curve entered in stage II, the fruits was resumed to rapid growth, the rapid growth rate of this stage is due to high transition of water and nutrients (sugar) from other parts of the plant to fruit (Abdulwahid 1998). After that, the fruit was entered the maturity then the ripening stage. The influence of water salinity on fruit weight has significant effect because the salinity effect on water relationship and all chemical process, which result an effect on fruit weigh (Abdulwahid et al 2016). Figure 2 showed pectin content decreased with the advancement of ripening in both treatments. The rapid decline of pectin content was due to the increased activity of cell wall enzymes PE and PG (Figure 3, 4), which were works on pectin, this aspect was also confirmed by other researchers (Ali & Abu-Goukh 2005; Alebresam 2009). 16 dsm water salinity affected the enzyme activity through gene expression of PG enzyme, in addition, affecting the chemical substances of this enzyme (Le Gall et al 2015).

The activity of PG in the first part of stage III, may be due to climacteric peak of respiration and ethylene (Abbas & Fandi 2001a), this is a signal to start the activity of enzymes and fruit firmness was started and then subsequently decreased, thereafter we can find a relationship between cell wall enzymes and fruit ripening, the relationship of compatibility between polygalactronic PG and pectinesterase PE, as the enzyme PE active in the early green stages to remove methoxy groups found in pectin chains which hinder the work of the enzyme endo-PG (Zhao et al 2006), which was missing during the first stage. The effectiveness of both enzymes (PG and PE) can be observed in the yellow phase. Unlike the green phase, it has been observed in the maturity stage that PG enzyme activity rises to the peak. After that, the activity of PG and PE declines when the ripening stage isinstalled (Peng et al 2002), Therefore we can say that the enzyme PE has an indirect role in firmness and maturity of fruit, while the PG enzyme has a direct role in it. This compatibility between PE work and PG in fruit is controlled by genetic programming of the fruit (Abdulwahid 2011), means when the fruit reaches the suitable age, some genes are activated then send the signal from nucleus to the cytoplasm to promote the synthesis of some proteins or enzymes which contribute to the ripening processor. The cellulase activity of the enzyme at the end of the stage II had no

significant level between the 4 dsm and 16 dsm treatments. It is possible that enzyme has a role in ripening processes, perhaps in conjunction with polygalacturonase (Liu et al 2008) to work fruit firmness (Ali & Abu-Goukh 2005) suggested cellulose may act in tomato fruit in conjunction with pectin enzymes to cause softening.

Conclusions. In conclusion, PE and Cx enzymes showed no significant difference between 4 dsm and 16 dsm treatments, PE is a basis of an action for PG enzyme, in addition, the fruits ripening is a genetically programmed process, with each enzyme's specific role. Therefore, we can conclude that there is a certain mechanism that may be genetically controlled on the action of cell wall enzymes which prevent deterioration or decline in the enzymes levels, which exhibit a significant difference in the enzymes of PE and Cx during the maturity, so we did not noticed a difference in ripening percent and only decrease in fruit weight.

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