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ORIGINAL ARTICLE

Olive Growth ,Carbohydrate, Mineral and Genomic DNA characterization as Influenced by Water Intensity

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ABSTRACT
Olive is an important and prime fruit crop in Middle East countries. It is considered as medicinal and nutritional fruit. The study was carried out to evaluate the olive growth, carbohydrate (glucose and inverted sugar) content and genomic DNA characterization as affected by water intensity shown as stress. Ten years old olive trees were used. The treatments were employed as watering at one week interval, four weeks interval and no watering (control) at all. The results showed that fruit diameter was higher in the trees watered once per week than the trees watered once at 30 days interval and in the trees no watered. Accordingly, per fruit weight was found higher watered once per week than watered once at 30 days interval and no watered treated fruit. In addition to that inverted sugar and glucose content were higher in the treated fruit watered at one week interval than 30 days interval and no watered treated fruit. Nutrient contents like NO_3^-, K^+, Ca^{++} and Na^+ were found higher in the trees watered at one week interval and 30 days interval than in the trees no watered. Moreover, K^+ content was higher in the case of all treatments compared to the other nutrient content. The highest acidic (lower pH) condition (sour) was found in no watered treated fruit. In addition, DNA band was wider in the trees treated having watered at one week interval than the trees 30 days interval and in the trees no watered. Therefore, our results suggest that watering to the olive trees from April to September once per week is the best for olive production and quality development.
Keywords: *olive, water stress, sugars, nutrient content and DNA band*

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INTRODUCTION

Olive (*Oleaeuropaea*) is a significant fruit crops in the Middle East countries as well as worldwide. It has major agricultural importance in the Mediterranean region as the source of olive oil [1, 2]. Saudi Arabia produced 20% production of olive in the world demand [3, 4]. Olive oil is increasingly present on the food scene as the healthiest alternative to other edible oils. Some studies indicate that olive oil helps to reduce the levels of low density lipoprotein [5]. Water is a significant factor for olive production and regeneration. Olive tree is highly resistance to drought where rainfall is limited [6,7]. However, irrigation increases the production of olive. Sufficient water increases shoot growth, flowering, fruit set and reduces fruit drop [7, 8,9,10.11.12]. A good water supply is also very important for obtaining satisfactory fruit size of table olives, which is a factor strictly correlated to the commercial value of the product [6,8,9,11,14]. Many trials have been carried out to determine the influence of water stress on the growth and production of olive, but little information is available on the relationship between irrigation and fruit quality of table olives [6, 15-17]. It was reported that yield of the 100% water application in olive trees was 74% higher than the rain fed treatment: 23.4 kg/tree (5.5 t/ha) vs.13.4 kg/tree (3.2 t/ha). The oil content also increased with the increase in the quantity of water applied from 39 to 53%. The 100% fruit weight was 58% higher in the full irrigation compared to no irrigation (171 vs. 108 g). Maturity of the fruit was accelerated with dryness (water stress of the trees). They also stated that mature rain fed olive orchards could be successfully converted to drip irrigation, resulting in a substantial yield increase and more than doubling the oil yield [17].

It was reported that table olives *(Olea europaea)* Irrigation every 15 days in table olive experimental garden from the end of June to mid September, induced higher leaf surface area, photosynthesis, and transpiration during the entire growing period compared to the control [18]. This led to an overall positive effect on total production per tree. Fruit weight, volume, and pulp/pit ratio all increased. Water availability influenced cell division more than cell expansion. They also reported that cell diameter, starch content, oil and chlorophyll content were higher in the irrigated trees that the nonirrigated trees [19].It was suggested that fruit diameter (2 cm), yield and per fruit weight (4.4 g) were increased with increasing the water volume (decrease the irrigation deficit) application. Water increases fruit quality and develop the fruit properly when there is a water stress condition plant may die and fruit growth may reduce. There are a few literatures regarding this, but no literature found in Saudi Arabia that work was done on this topic. That is why it is an important issue in KSA. Objectives of the experiment were to know the effect of water intensity (stress)on fruit growth, biochemical content like pH, sugar and glucose content as well as DNA band (fingerprint/profile) of olive fruit.

MATERIALS AND METHODS

Materials

Ten years old olive trees were selected from olive garden, Hail, Saudi Arabia. Watering tools (hose pipe) were used.

Methods

Fifteen olive trees were selected from the garden located in Hail, KSA. Five trees were employed for the treatment of one week interval watering (one week water stress), five trees were selected for the treatmentof one month interval watering (30 days, [four weeks] water stress) and five trees were selected for the treatment of no watering (Control). Treatment was set before flowing in May. There were five replicated trees in each treatment. Five uniform (age, length [for all branches 20 cm from top], diameter, light intensity) branches were selected in each tree. So total of replicated branches was 25 for each treatment. (Figure 3)

Data Collection

Flower and fruit set, Fruit growth and size (length and diameter) were measured. Fruits were harvested from each trees in the last of September and weighed. Per fruit weight,. Fruit were ground with motor and pestle and finally extracted juice was collected and kept in the freezer for short time.

Data analysis

Biochemical (glucose and inverted sugar) content was determined. Finally DNA isolation, quantification and sequencing were done.

Glucose content determination

Glucose was determined by using glucose refractometer. 3 drops of juice sample were put on the disc of the meter and data were displayed and recorded (Figure 4).

Inverted sugar determination

Inverted sugar was determined by using inverted sugar refractometer. Threedrops of juice sample were put on the disc of the meter using small syringe dropper and data were displayed and recorded.

Nutrient content determination

Nutrient content (NO_3, K and Ca) was determined by using Horiba NO3, K and Ca meters (USA) (fig. 2). 5 drops of juice sample were put on the disc sensor of the meter using small dropper and data were displayed and recorded (Figure 4).

DNA isolation

5ml CTAB was preheated (added 10µl mercaptoethanol to each 5ml CTAB) in a blue-topped 50ml centrifuge tube at 60-65^{o}C. Skin was separated and wrapped with aluminium foil and freezed in liquid nitrogen. Sample (1.0 g tissue/5ml CTAB)was stored after liquid Nitrogen 2 days at –20^{0}C.Fruit tissue was crumbled over cold pestle of liquid nitrogen. Ground frozen fruit tissue with one spatula of fine sand added 0.5 spatula of PVPP powder after grinding. Scraped powder into dry tube and added pre-heated buffer and mixed gently. CTAB volume was adjusted to give a slurry-like consistency, mix occasionally. It was incubated for 60 min at 60 o C. Equal volume of chloroform/iso-amyl alcohol (24:1) was added and mixed for about 3min, then transferred contents to narrow bore centrifuge tubes. It was balance by adding extra chlor/iso. It was made spin 5,000rpm for 10min and broken off. Supernatant was removed with wide-bore paste (cut off blue tip) to clean tube, repeat chloroform extraction once. Supernatant should be clear, though may be colored. Precipitated DNA with 0.66 vol. of cold isopropanol and left overnight. Spooled out or spin down DNA, 2min at 2,000rpm. DNA sample was transferred to 5ml wash buffer for 20min. it was dried briefly and re-suspended in 1ml T.E. (can be left overnight). 1µl 10mg/ml RNAse was added to each 1ml T.E./DNA mixture and incubated for 60min at 37^{o} C. It was diluted with 2

volumes TE and added 0.3vol 3M sodium acetate [(pH 8) + 2.5 vol cold 100% ethanol].Spooled DNA was taken out, air dried and re-suspended in 0.5 to 1ml TE or water and it was freezed until required.

DNA Quantification and characterization by agarose-gel electrophoresis

DNA weight was measured by electric balance using eppendrop tubes.

Materials

Electrophoresis box, Power supply, Gel tray and comb, micropipette, loading dye, agarose, 1X TBE buffer, ethidium bromide, transilluminator/camera, 1.5 ml eppendrof tubes.

Method of DNA characterization

A 0.8% agarose gel was made with 99.2% 1x TAE and 0.1µl of Ethidium bromide (10mg/ml) per 10ml solution. Load samples undiluted and at a 1 in 10 (1+9) dilution with 3µl loading buffer. Incubated overnight at room temperature or 2 hours at 38 0C.It was loaded 1 ul of loading dye into each sample. Adjusted the micropipet to 11 ul and load the samples in lanes 2-8. In lane one added the Lambda/HindIII digestion (10ul of 0.1 ug/ul sample for a total of 1ug of DNA) plus one ul of loading dye (11 ul). It was run at 90-100 volts for one hour. The gels were stained for approximately 5 minutes in ethidium bromide and de-stain in water for 2 minutes. Photograph was taken for gels. DNA molecules are negatively charged due to dissociation of the phosphate backbone. During electrophoresis they migrated towards the positively charged electrode. Small DNA fragments migrated more rapidly in the gel matrix compared to large fragments, resulting in molecule separation based on size.

Statistical Analysis

Data were analyzed statistically. Standard error (SE) and Least Significant difference Test (LSDT) were employed.

RESULTS AND DISCUSSION

In figure 1, it has been shown fruit diameter was higher in the treated fruit (watering once per week and once at 4 weeks) than the no watered olive fruit (control). Accordingly it has been shown in figure 2 that fruit weight was higher in the treated fruit (watering once per week and once at 4 weeks) than the no watered olive fruit (control). It might be due to the water availability in the treated trees. It might be the effect of water stress on photosynthates. Water stress closes the stomata in chloroplast and reduces the penetration of CO_2 and finally photosynthetic yields (carbohydrate, sugars etc.) are affected.

[19]It was reported that under water stress conditions, cell elongation of higher plants can be inhibited by the stress of water flow from the xylem to the surrounding elongating cells. He also mentioned that growth inhibition might occur at low water potentials and it could be reversed by increasing the xylem water potential by means of water availability using pressure application in the root region [19, 20]. It was suggested that fruit diameter and per fruit weight were increased with increasing the water volume (decrease the irrigation deficit) application.

Inverted sugar and glucose content were found to be higher in the treated fruit (watering once per week and once at 4 weeks) than the no watered olive fruit (control) [Table 1]. It may be due to the water condition [21]. It was stated that an adequate water supply prevented the plants from wilting. He also described that this was not only happened to water levels but also plant pigment (biochemical process) that was important for photosynthesis. It is the biochemical process by which plants produce food for growth through the use of light and water. He also suggested that carbohydrates were derived from carbon dioxide and water during the process.

Acid content in fruit was lower (represented by pH higher) in the treatment where watered weekly than other treatments (watered once a month) and control (no water)(Table 1). It might be due to the effect of water stress on photosynthetic yield. Usually there are the more carbohydrate, the more alkaline condition (less acid) and the less acid (more pH) [4].It was reported that the water content was affected the sugar TSS and titratable acidity of peach trees and physiological stress affected the chlorophyll content in peach trees.

Nutrient contents like NO_3, K, Ca and Na were higher in the trees watered at one week interval and 30 days interval than in the trees no watered. Moreover, K content was higher in the case of all treatments compared to the other nutrient content (Table 2). This may be the effects of water quantity in different trees. DNA band/probe was wider in the trees treated having watered at one week interval and the tree. s 30 days interval than in the trees no watered (Figure 5). This may be the effects of water deficiency or hydrophobic effect. The hydrophobic effect may responsible for the stability of cell membranes, drives protein folding as well as the insertion of membrane proteins [22]. It was reported that estimated 20–30% of all genes in most genomes encode membrane proteins [22].

CONCLUSION

Our results conclude that that watering to the olive trees from April to September once per week is the best for olive production and quality development. It (per week watering) is more effective for fruit growth, biochemical content like pH, sugar and glucose content, nutrient content as well as DNA band (fingerprint/profile) of olive fruit.

Table 1.Biochemical content determination at different treatments.

Treatment	Inverted Sugar (%)	Glucose (%)	pH
Water once/ week	9.4±0.04	9.2±0.2	4.2±0.02
Water once/month	8.2±0.05	7.5±0.04	3.6±0.02
No water	5.1±0.03	4.4±0.02	2.6±0.02

Mean±SE (n=5)

Table 2. Nutrient content determination at different treatments

Treatment	NO^{3-} ppm	K^{+} ppm	Ca^{++} ppm	Na^{+} ppm
Water once/ week	242±0.3	610.5±o.2	35.5±0.1	78.4±0.2
Water once/30 days	222±0.2	580±0.3	33.2±0.2	70.3±0.1
No water	182.1±0.2	496.2±0.3	28.3±0.1	55.2±0.1

Mean±SE (n=5)

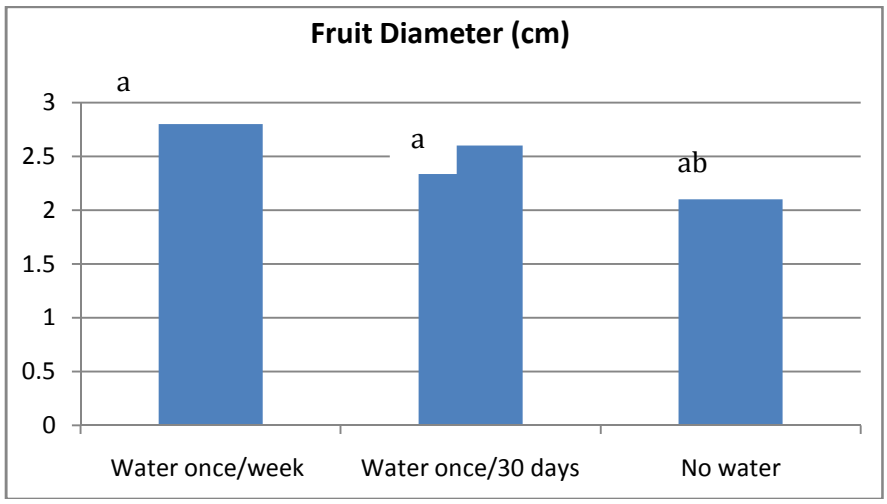


Figure 1. Olive fruit diameter as affected by different treatments. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean±SE (n = 5).

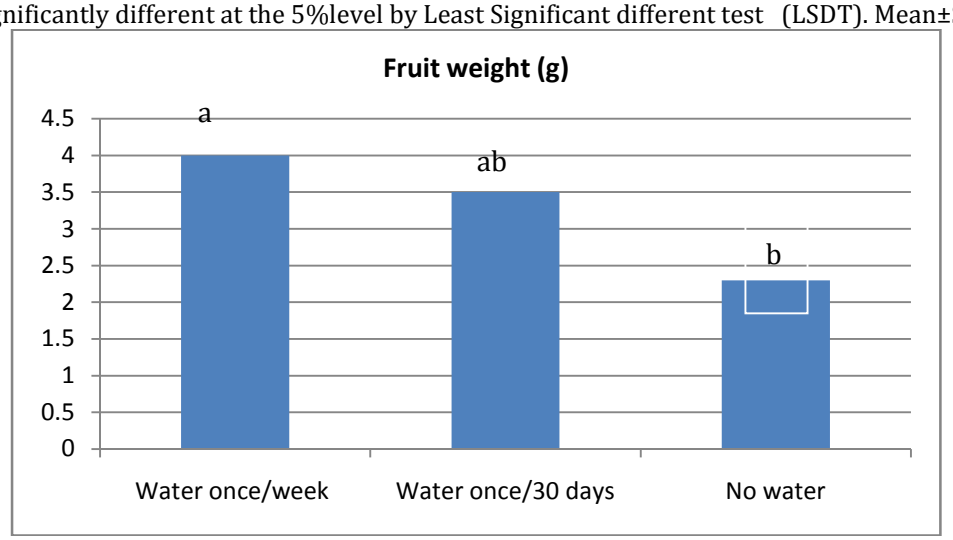


Figure 2. Olive Fruit weight as affected by different treatments. Means followed by the common letters are not significantly different at the 5%level by Least Significant different test (LSDT). Mean±SE (n = 5).

Water once/per week Water once (after 30 days)No water (depending on rain)
(Treatment at different trees)

Water once/per week Water once/30 days No water

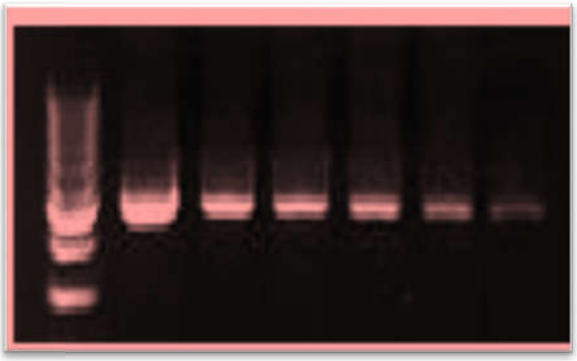
a. Fruit structure in different treatments

b. Grinding process. Extracted juice

Figure 3. Photograph shows the treated plant and juice extraction process

a. Glucose meter b. NO_3 meter (Horiba Scientific, USA)

Figure 4.Photograph shows the glucose and nutrient measurement process



S 1 2 3 1 2 3

Figure 5. Identification of DNA band (DNA Ladder/ Probe) in gel electrophoresis. 1 = Water once/per week (3 replicates), 2 = Water once (after 30 days) (3 replicates), 3 = No water (depending on rain) (3 replicates).

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