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Growth and Productivity of Pomegranate Trees under Different Irrigation Levels. III: Leaf Pigments, Proline and Mineral Content

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Abstract: This investigation was carried out during 2007 and 2008 seasons on trees of Manfalouty pomegranate cultivar (*Punica granatum. L*). The trees were grown at El-Kassasien Research Station, Ismailia Governorate in sandy soil under drip irrigation system. The trees received the following five irrigation levels: 7 or 9 or 11 (control) or 13 and 15m^3/tree/season. The data indicated that, the reduction in irrigation rate was concurrent with decrease in chlorophyll a, b and carotenoids in both seasons. Irrigation level at 7m^3/tree/season gave the highest significant leaf proline, while the farm control (11m^3/tree/season) showed an intermediate leaf proline. The lowest significant proline was resulted from the highest irrigation level (15m^3/tree/season). Leaf nitrogen percentage increased by increasing irrigation level from 7 m^3/tree/season to 13m^3/tree/ season, but a slight reduction in N percentage was observed by increasing irrigation rate to 15m^3/tree/season. The highest leaf P percentage was recorded by irrigation at 13m^3/tree/season sequenced by 15m^3, farm control 11m^3 then 9m^3/tree/season. Meanwhile, irrigation at 7m^3/tree/season gave the lowest significant value of P percentage. As the level of irrigation supply increased, a subsequent increment was observed in plant K percentage. Ca percentage increased by increasing irrigation levels, the highest significant Ca-percentage was recorded with highest irrigation level (15m^3/tree/season) followed by 13, 11 then 9m^3/tree/season. On the other hand, the lowest Ca-percentage was recorded by irrigation level 7m^3/tree/season.

Key words: Pomegranate · Irrigation levels · Chlorophyll · Carotenoids · Proline · N · P · K · Ca

INTRODUCTION

The pomegranate (*Punica granatum* L.) is a popular fruit of tropical and subtropical regions, belonging to the family punicaceae and it is one of the oldest cultivated fruit plants. Manfalouty pomegranate is considered one of the most important cultivars grown successfully in Egypt. Pomegranate trees are amenable to irrigation with saline water and fairly drought resistant but require normal watering for good growth and productivity. There is an increasing demand on water resources used for irrigation and need to maximize agriculture water utilization efficiency. The amount and quality of available irrigation water of the arid and semi- arid regions of the world such as Egypt, are the main limiting factors for extension agriculture. Therefore plant growth and development retarded when water supply was restricted. There are many opinions about the effect of irrigation water amounts on nitrogen content of plant. Some studies showed a linear relationship while others revealed the opposite trend. Also, there is a general significant positive effect on the percent of P and K in pomegranate leaves due to increasing available soil water [1, 2]. Also, leaf chlorophyll and carotenoids concentration of pomegranate seedling decreased significantly by decreasing available water [2, 3].

Proline accumulation may be an indicator of drought resistance. There is extensive literature indicating that, drought stress induces an accumulation of proline suggesting that amino acid may be a useful early indicator of drought stress effects, although there is evidence in the literature that proline accumulation seems to occur only when plant growth is already retarded by drought stress [4-6]. Verranjaneyulu and Kumari [7] found that, proline accumulation occurred in roots and leaves, where enzymes proline dehydrogenase and proline oxidase were

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inhibited under water stress. Also, Shawky *et al*. [8] indicated that, a high degree of water stress reduced plant growth and at the same time leaf proline percentage increased.

Therefore, this experiment was designed to study the effect of different irrigation levels on pigments, proline, N, P, K and Ca in leaves of manfalouty pomegranate cultivar.

MATERIALS AND METHODS

Field experiment on 20 year old mature pomegranate trees (*Punica granatum* .L) Manfalouty cultivar was conducted during two successive seasons of 2007 and 2008 at El- Kassasien Research Station, Ismailia Governorate. Trees distances were of 5 meters between trees and between lines. Trees received the recommended horticulture management of the Horticultural Research Institute (H.R.I.). Uniform fifteen trees were selected randomly where the experiment included five irrigation levels: 7 or 9 or 11 (control) or 13 and 15m^3/tree/season. Each treatment was replicated three times with one tree for each replicate and the randomized complete blocks design was used. The daily amount of irrigation water as liters per tree for each treatment in 2007 and 2008 seasons are shown in Table (1).

Data Recorded

Leaf Chemical Analysis: Leaves were sampled from middle of shoots of average length and well exposed to light, taken in July of both seasons according to Chandler [9] to determine the following:

Pigments Concentration: Samples of mature fresh leaves were hemogenized with acetone (85% V. V) in presence of Na_2CO_3 and silica quartz; then filtered through central glass funnel G4, the residue was washed several times with acetone until being free from pigments. Each filtrate was made up to 25 ml and measured colourimetricaly at wave length of 662, 644 and 440.5 n m to determine chlorophyll a, b and carotenoids, respectively according to Brougham [10]. The concentration of pigment was calculated from the following equations

$$\text{Chlorophyll a (mg/gm)} = \frac{9.784 \text{ x } 662 - 0.99 \text{ x } 644}{\text{A x W x l00}} \text{ x V}$$

$$\text{Chlorophyll b (mg/gm)} = \frac{21.428 \text{ x } 660 - 4.65 \text{ x } 662}{\text{A x W x l00}} \text{ x V}$$

$$\text{Carotenoids (mg/gm)} = 4.695 \text{ X } 440.5 - 0.268 \text{ x (chl a + chl b)}$$

Where:- V: is the volume in ml. A: is the length of light path in the cell. W: is the fresh weight in grams. 662, 644 and 440 are the absorbency of chlorophyll a, b and carotenoids

Leaf Mineral Percentage: The samples of leaves were oven dried at 70°C for 48 hours (until a constant weight) then grounded and used in preparing the wet digested solution (1:4 perchloric acid : sulphuric acid) as described by Piper [11] which analyzed for total macro elements.

Total Nitrogen (%): Was determined by the modified micro-keyldahl method [12].

Phosphorus (%): Was estimated coloremetically (ammonium molybdate) according to the official methods of analysis [13].

Potassium (%): Was determined by using flame photometer [14].

Calcium (%): Was determined by atomic absorption spectrophotometer Perkin Elmer-3300 [15].

Proline Percentage: Mature fresh leaf samples (0.5 gm) were homogenized in 10 ml 3%-5% sulphosalisylic acid then filtered through Whatman No.1 filter paper. The filtrate (2 ml) was added to 2 ml ninhydrin reagent and 2 ml glacial acetic acid and then the mixture boiled on water

Table 1: Distribution of the irrigation water (L/day/tree) through the two seasons of study (2007 and 2008).

	Month (2007 and 2008)								
Treatments	Mar.	Apr.	May	Jun	Jul.	Aug.	Sep.	Oct.	Nov.
7m^3/tree/season	4	10	28	50	50	50	28	10	4
9m^3/tree/season	6	15	40	60	60	60	40	15	6
11m^3/tree/season	8	21	50	70	70	70	50	22	8
13m^3/tree/season	12	26	60	80	80	80	60	26	10
15m^3/tree/season	14	30	65	95	95	95	65	30	12

both for one hour. The boiled mixture was put in ice both, then 4 ml were added to each sample with severely inverting, then colourimetrically estimated at 520 nm according to Bates *et al*. [16]. The proline concentration was determined from standard curve and calculated on fresh weight basis. The obtained data were tabulated and statistically analyzed according to Snedecor and Cochran [17]. Differences between means were compared by Duncan's multiple range test at 5% level of probability according to Duncan [18].

RESULTS AND DISCUSSION

Leaf Pigments Concentration: In both seasons of the study similar trend was observed for leaf pigments (chlorophyll a, b and carotenoids) under different irrigation treatments (Table 2). The data indicated that, the reduction in irrigation rate was concurrent with decrease in chlorophyll a, b and carotenoids in both seasons. This increment in leaf pigment concentration could be attributed to increasing of macronutrient uptake, especially N and Mg as a consequence of improved soil moisture under irrigation.

The same phenomenon was also described by Abou El-Wafa [3] on pomegranate, Hassan [19] on olive, pomegranate, lemon and almond and Fathi [20] on pear they found that, maximal and variable chlorophyll were significantly reduced by the drought stress. In addition, photosynthetic pigments content in leaves was significantly higher in the Canino apricot and Anna apple trees grown under high irrigation rate [21, 22].

Leaf Proline Percentage: Data of leaf proline percentage in Table 2 indicated that, reducing water level significantly raised the concentration of proline. Irrigation level at $7m^3$ gave the highest significant leaf proline (0.96 and 1.20%), while the farm control ($11m^3$) showed an intermediate leaf proline (0.68 and 0.85%). The lowest significant proline (0.31 and 0.51%) was resulted from the highest irrigation level ($15m^3$).

These results are in harmony with the conclusion given by Verranjaneyulu and Kumari [7] who found that, proline accumulation occurred in roots and leaves, where enzymes proline dehydrogenase and proline oxidase were inhibited under water stress. Leaf free proline percentage of Canino apricot and Anna apple was significantly high under least irrigation treatment [21, 22]. So, the relationship between water irrigation level and the accumulation of proline in the leaves could be explained as water stress inhibit proline oxidation in the mitochondria and alter the permeability of the mitochondrial membranes. Furthermore, the incorporation of proline into protein is inhibited by waters stress, thereby also leading to proline accumulation under stress conditions. Proline accumulation was observed when protein synthesis inhibitors, such as cycloheximide. Also, proline greatly increased the amount of bound water in leaves compared with other protein forming amino acid [23]. Proline accumulation may be an indicator of drought resistance [4]. Also, accumulation of proline in the cytoplasm also plays an important role in osmotic adjustment in plants [24].

Leaf Mineral Percentage

Nitrogen (N%): Data in Table 3 showed that, leaf nitrogen percentage increased by increasing irrigation level from 1.80 and 1.72% by irrigation with 7 m^3/tree/season to 2.10 and 2.03% with $13m^3$/tree/ season, but a slight reduction in N (1.95 and 1.90%) was observed by increasing irrigation rate to $15m^3$/tree/season in both seasons.

Phosphorus (P %): Data in Table 3 indicated that, the highest leaf P percentage (0.27 and 0.25%) was recorded by irrigation at $13m^3$/tree/season sequenced by $15m^3$ (0.27 and 0.23%), farm control $11m^3$ (0.25 and 0.23%) then $9m^3$ (0.23 and 0.22%) however the differences were insignificant. Meanwhile, irrigation at $7m^3$/tree/season gave the lowest significant value (0.22 and 0.20%) in both seasons.

Potassium (K%): Data in Table 3 revealed that, irrigation level at $11m^3$/tree/ season produced K 1.58 and 1.44%. With decreasing irrigation levels to 9 then $7m^3$, K was decreased to 1.52, 1.30 and 1.45, 1.34% respectively in both seasons. On the other hand, irrigation level at 13 and 15 m^3/tree/ season increased K percentage (1.66, 1.50 and 1.61, 1.44%) in both seasons. Generally, as the level of irrigation supply increased, a subsequent increment was observed in plant K percentage.

Calcium (Ca %): Data in Table 3 appeared that, Ca percentage increased by increasing irrigation levels, the highest significant Ca-percentage (1.63 and 1.60%) was recorded with highest irrigation level ($15m^3$) followed by 13, 11 then $9m^3$/tree/season. While, the lowest value (1.30 and 1.18%) was recorded by irrigation level $7m^3$.

Table 2: Effect of irrigation levels on leaf pigments concentration and proline (%) of pomegranate cv. Manfalouty in 2007 and 2008 seasons.

Irrigation levels (m^3/tree/ season)	Chlorophyll a (mg/g F.W)	Chlorophyll b (mg/g F.W)	Carotenoids (mg/g F.W)	Proline (%)
2007 season				
$7m^3$	0.85 e	0.46 e	0.42 c	0.96 a
$9m^3$	0.93 d	0.51 d	0.48 c	0.75 b
$11m^3$ (control)	1.01 c	0.57 c	0.55 b	0.68 b
$13m^3$	1.20 b	0.63 b	0.65 a	0.44 c
$15m^3$	1.29 a	0.71 a	0.68 a	0.31 d
2008 season				
$7m^3$	0.84 d	0.43 c	0.42 d	1.20 a
$9m^3$	0.91 cd	0.47 c	0.44 cd	0.98 b
11 m^3 (control)	0.93 c	0.53 b	0.48 c	0.85 c
$13m^3$	1.11 b	0.59 a	0.57 b	0.64 d
$15m^3$	1.23 a	0.65 a	0.64 a	0.51 e

Values followed by the same letter (s) in each column are not statistically differed at 5% level

Table 3: Effect of irrigation levels on leaf mineral percentage of pomegranate cv. Manfalouty in 2007 and 2008 seasons.

Irrigation levels (m^3/tree/ season)	N (%)	P (%)	K (%)	Ca (%)
2007 season				
$7m^3$	1.80 c	0.22 b	1.45 d	1.30 d
$9m^3$	1.83 c	0.23 ab	1.52 c	1.37 cd
$11m^3$ (control)	1.88 bc	0.25 ab	1.58 bc	1.45 bc
$13m^3$	2.10 a	0.27 a	1.66 a	1.51 b
$15m^3$	1.95 b	0.27 a	1.61 ab	1.63 a
2008 season				
$7m^3$	1.72 c	0.20 b	1.34 b	1.18 c
$9m^3$	1.76 bc	0.22 ab	1.30 b	1.22 c
$11m^3$ (control)	1.81 bc	0.23 ab	1.44 a	1.43 b
$13m^3$	2.03 a	0.25 a	1.50 a	1.52 ab
$15m^3$	1.90 ab	0.23 ab	1.44 a	1.60 a

Values followed by the same letter (s) in each column are not statistically differed at 5% level

These results are confirmed with the results obtained by Ibrahim and Abd El-Samad [1] on pomegranate; Abd El-Nasser and EI-Shazly [25] and Mikhael [22] on apple they mentioned that, there is a general significant positive effect on the percent of N, P and K in leaves due to increasing available soil water. Similarly, Khalil [26] on olive found that, K content in leaves was significantly reduced by decreasing irrigation rate. Channel and Ranbirsingh [27] on Dashehari mango and Ahmed [28] on pomegranate trees indicated that, leaf content of Ca was greater with increasing irrigation levels.

These results may be led to the conclusion that nutrient uptake was retarded under water stress condition where a substantial decrease in transpiration rates and impaired active transport and membrane permeability and resulting in a reduced root absorbing power of plant. So depletion of soil moisture level caused a reduction in leaf mineral content.

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