

RESPONSE OF WATER REQUIRMENT AND LECTTUCE YIELD TO WATER STRESS AND PROLINE FOLIAR UNDER DRIP IRRIGATION SYSTEM

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Abstract: A field experiment was conducted to grow lettuce (*Lactuca sativa* L.) during the 2021-2022 season in Al-Yusufiyah district, south of Baghdad, to study the effect of irrigation levels and spraying with proline acid on water requirements, growth and production of lettuce. The experiment was designed using a complete randomized block design (RCBD) and included two factors: the first factor (irrigation levels) includes three treatments (irrigation after depletion 25, 40 and 55% of the available water) and the second factor (concentrations of adding proline acid sprayed on the leaves) includes three treatments (not adding proline and adding proline sprayed on the leaves at a concentration of 40 and 80 mg L⁻¹). The results of the study were as follows: Seasonal water consumption reached 262, 233 and 186 mm for the moisture depletion treatments of 25, 40 and 55% of the available water, respectively, under the drip irrigation system. The total vegetative yield of lettuce was 247.67, 215.10 and 171.73 µg ha⁻¹ at 25, 40 and 55% depletion levels of available water, respectively. The total yield of lettuce was 258.30, 228.50 and 181.40 µg ha⁻¹ at 25, 40 and 55% depletion levels of available water, respectively, when spraying proline acid at a concentration of 80 mg L⁻¹.

Keywords: water consumptive use, moisture depletion, lettuce, drip irrigation

INTRODUCTION

Understanding the water requirements of all field and horticultural crops and increasing water productivity is particularly important, especially for leafy vegetable crops, which are among the most produced and consumed vegetables worldwide due to their essential nutrients in the human diet. To compensate for the shortage in water resources, several methods are used to ensure water reaches agricultural lands and plants while minimizing water loss. This can be achieved using surface and subsurface drip irrigation systems. The key justifications for adopting drip irrigation systems include reducing irrigation water consumption, minimizing labor efforts, decreasing water wastage, lowering irrigation costs, and improving and increasing crop productivity. Therefore, increasing water productivity and crop yield per cubic meter of water plays a crucial role in mitigating water scarcity issues. Water is a vital agricultural input in arid and semi-arid regions, where it significantly impacts agricultural production. Hence, irrigation management and improving irrigation systems are of paramount importance for enhancing water productivity (2, 3, 5, 24). Water resources, in addition to their impact on the quality and quantity of production, serve as the primary factor determining agricultural production and expansion, especially in arid and semi-arid regions. The agricultural sector is the largest consumer of water. Efforts are focused on optimizing water use irrigation by employing modern agricultural and irrigation techniques that enhance water efficiency to achieve high returns and maximize the yield per unit of agricultural water (20). Therefore, it is essential to safeguard water sources, prevent waste, and regulate water use to attain maximum productivity with minimal water consumption. This can be achieved through agricultural practices aimed at mitigating physiological stress symptoms that occur in plants due to harsh environmental conditions such as drought. One such practice involves foliar spraying of proline, as numerous studies have demonstrated that proline plays a significant role in plants by enhancing tolerance to adverse conditions such as heat, cold, drought, and salinity. Proline contributes to increasing the protoplasm content of cells, thereby reducing damage caused by stress. Additionally, proline accumulation in plant tissues enhances membrane flexibility, maintaining cell turgor and preserving enzymatic activity. Proline improves plant growth by forming hydrogen bonds, stabilizing proteins within the plant, and maintaining membrane integrity (13;31). Furthermore, proline protects cells by enhancing their ability to absorb water and

activating enzymes. It is also recognized as an antioxidant compound (10, 11). (12) reported that foliar application of proline could improve the productivity and quality of lettuce under water stress conditions.

Lettuce (*Lactuca sativa L.*), part of the Asteraceae family, is an important source of antioxidants and phytochemicals with cancer-fighting properties. It is rich in vitamins A, E, and C, along with various minerals. Additionally, lettuce contains low amounts of fat, dietary fiber, protein, and carbohydrates (1, 15). Its cultivated area covers approximately 1.3 million hectares, with a global production of 29 million tons, making it one of the most widely grown leafy vegetables (14). Deficit irrigation is an essential strategy for water conservation in arid and semi-arid regions with limited water resources. Significant improvements in water productivity have been achieved by regulating water stress through controlled irrigation amounts. Therefore, determining crop water requirements is critical, especially in regions with low rainfall, as water is a key factor in crop production. This study was conducted to determine the water requirements of lettuce under different levels of soil moisture depletion and foliar application of proline, as well as to evaluate its growth and productivity parameters.

MATERIALS AND METHODS

Experimental Site and Pre-Planting Soil Characteristics

The field experiment was conducted in Baghdad Governorate, Mahmoudiyah District - Yusufiyah Subdistrict during the 2021-2022 growing season to study the effects of irrigation levels and foliar application of proline acid on water requirement, growth, and yield of lettuce. The experiment was conducted on a silty clay loam, classified under the Typic Torrifluvent subgroup according to the classification system. Before planting, soil samples were collected from various locations in the field at a depth of 0–30 cm, then mixed to obtain a composite sample. The sample was air-dried, ground, and sieved through a 2 mm mesh sieve, followed by physicochemical analyses (Table 1). The experimental field plowed perpendicularly, then leveled, adjusted, and finely tilled. The land was divided into experimental units, with a total area of 15 m².

Table 1. Some Physical and Chemical Properties of the Soil Before Planting

Property	Units	Soil Depth (0.0–0.30 m)	Method of Measurement and Source
Sand		374	
Silt	g kg ⁻¹ soil	261	
Clay		365	Pipette Method (8)
Soil Texture Class	Silty Clay Loam		
Bulk Density		1.32	Core Method according to Hartge and Blake as described in (23)
Particle Density	µg m ⁻³	2.64	Method Pycnometer
Volumetric Water Content at kPa		0.377	
Volumetric Water Content at kPa	cm ³ cm ⁻³	0.198	Pressure Plate Apparatus method
Available Water		0.179	
EC	dS m ⁻¹	2.46	
pH		7.7	Saturated Paste Method as reported in (7)
Organic Matter	g kg ⁻¹ soil	16.8	Method according to Wakely and Black as described in (21, 25)
Available Nitrogen		41.88	Bremner and Keeney Method, Olsen and
Available Potassium	mg kg ⁻¹ soil	92.88	Sommers, Flame Photometer Method as

Planting and Irrigation System

Lettuce seeds (Fajr variety) were obtained from the Iraqi Ministry of Agriculture and sown in a nursery on September 20, 2021. After one month, when the seedlings reached the 2–3 true-leaf stage, they were transplanted to the field on October 21, 2021. Immediately after transplanting, seedlings were irrigated using drip irrigation systems. The row spacing was 40 cm, while the plant spacing within rows was 30 cm.

Fertilization Management

The experimental field was fertilized with 200 kg ha⁻¹ of superphosphate and 200 kg ha⁻¹ of urea fertilizer. The phosphorus fertilizer was applied before transplanting during soil preparation, whereas urea was applied in two splits: First dose: 100 kg ha⁻¹, three weeks after transplanting. Second dose: 100 kg ha⁻¹, at the heading stage (26).

Harvesting

Lettuce heads were manually harvested on January 25, 2022. Figure 1 presents some field application images illustrating the experimental process.

Experimental Design and Treatment Factors

The experiment was designed using a Randomized Complete Block Design (RCBD) and included two factors:

The first factor (Irrigation Levels) consisting of three treatments:

I1: Irrigation after depleting 25% of the available water.

I2: Irrigation after depleting 40% of the available water.

I3: Irrigation after depleting 55% of the available water.

Second factor (Proline foliar application concentrations) consisting of three treatments:

B0: No proline application.

B1: Foliar application of proline at 40 mg L⁻¹ (twice).

B2: Foliar application of proline at 80 mg L⁻¹ (twice).



Figure 1. Some Applied Images of the Fieldwork.

As a result, the total number of experimental units was 27 (3 irrigation levels $\times$ 3 proline concentrations $\times$ 3 replicates). The experiment was conducted following the Completely Randomized Block Design (RCBD) with three replicates, and treatments were randomly assigned. The Least Significant Difference (LSD) test at $p < 0.05$ was used to compare the treatment means. Data analysis was performed using Genstat v12.1, and significant differences between means were determined using the Least Significant Difference (LSD) test.

Drip Irrigation System Calibration and Evaluation

Several tests were conducted to evaluate the emitter discharge rate and uniformity of water distribution in the surface drip irrigation system. One pipe from the replicates was selected for the surface drip irrigation treatment. Twenty plastic containers (each with a 0.15 m radius and 0.01 m height) were placed under the emitters to collect the water during system operation. The tests were performed at different operating pressures: 50, 75, 100, and 150 kPa, and pressure was measured using a mechanical pressure gauge with a capacity of 1000 kPa over a defined operating period.

The collected water volumes in each container were measured to determine the emitter discharge rate, expressed in L h^{-1} (liters per hour). The surface drip irrigation system was calibrated at an operating pressure of 50 kPa, as this pressure provided the best water distribution uniformity, the highest uniformity coefficient, the best irrigation application efficiency, the highest low-quarter distribution uniformity, and the lowest coefficient of variation.

Irrigation Scheduling and Water Consumption Calculation

The available water content was determined from the difference between the volumetric water content at 33 kPa, which represents the field capacity, and the volumetric water content at 1500 kPa, which represents the permanent wilting point, according to the following equation (4):

$$AW = \theta_{fc} - \theta_{wp} \quad (1)$$

Where:

AW = Available water content in the soil ($\text{cm}^3 \text{cm}^{-3}$).

θ_{fc} = Volumetric water content at field capacity ($\text{cm}^3 \text{cm}^{-3}$).

θ_{wp} = Volumetric water content at permanent wilting point ($\text{cm}^3 \text{cm}^{-3}$).

Calculation of Applied Water Depth

$$d = (\theta_{fc} - \theta_w) \times D \quad (2)$$

Where:

d = Applied water depth (mm).

θ_{fc} = Volumetric water content at field capacity ($\text{cm}^3 \text{cm}^{-3}$).

θ_w = Volumetric water content before irrigation, based on the moisture depletion level ($\text{cm}^3 \text{cm}^{-3}$).

D = Soil depth, which corresponds to the effective root zone depth (mm).

Growth and Yield Indicators

Head length (cm plant^{-1}): Measured from the stem-soil junction to the longest leaf in the head, and the average was calculated.

Head circumference (cm): Measured by wrapping a measuring tape around the widest part of the head, and the average was calculated.

Total head yield ($\mu\text{g ha}^{-1}$): Determined by measuring the total yield weight from the experimental unit, then converted to hectare yield.

RESULT AND DISCUSSION

Table 2 presents the effect of depletion levels on the seasonal water consumption of lettuce under the surface drip irrigation system. The results indicate that the seasonal water consumption at the 25% depletion level of available water (I1) was 98, 81, 58, and 25 mm for the seedling stage, vegetative growth stage, head formation stage, and maturity stage, respectively. At the 40% depletion level of available water (I2), the seasonal water consumption was 98, 68, 48, and 19 mm for the same growth stages, respectively. Meanwhile, at the 55% depletion level of available water (I3), the seasonal water consumption was 98, 42,

32, and 14 mm for the corresponding growth stages, respectively. The results demonstrate an increase in seasonal water consumption during the first growth stage (seedlings) due to the irrigation system's approach in which the first irrigation was applied up to field capacity at a depth of 30 cm. The subsequent irrigations were applied after depleting 35% of available water at a depth of 30 cm. This stage lasted for 20 days, requiring six irrigations. During the vegetative growth stage, the irrigation treatments were separated and applied after depleting 25%, 40%, and 55% of available water, with the applied water depth calculated accordingly. This phase lasted for 35 days, where seven irrigations were given. Results further show dwindling water intake during the head formation and maturity stages across the board on levels of irrigation. Such dwindling was noted toward the end of November, and since December was already seen, when air temperature began to reduce, there was a drop in evapotranspiration as well as that of the crop demand for water. The head formation stage took 19 days with three irrigations, and the maturity stage took seven days with one irrigation. The seasonal crop evapotranspiration increased with the duration of the growth period under all levels of irrigation reaching a peak during the vegetative growth stage. The rise is due to increased water requirements by the plant during this stage since there is rapid growth, increase in size of the plant, and physiological development along with the expansion of the root system thereby enhancing the efficiency of water absorption. The increase in leaf area also leads to increased transpiration losses. High temperatures and high intensity solar radiation further increase the loss of water, particularly through evaporation from the soil surface. All these add to a higher crop water requirement.

On the other hand, less water is consumed at the head formation and maturity stages as compared to the vegetative growth stage. The reduction comes because plant growth is finished, there is more leaf area, and ground cover which all decrease soil evaporation and thus the water needs of the plant. As the plant continues to grow, water consumption decreases, and it is at its minimum during the head formation stage of the crop because now the crop has fully entered its mature stage, and it has reduced its water requirements. Full maturity is characterized by full leaves and stems with the head developed, which falls simultaneously with low temperature as well as high relative humidity and short sunshine hours, all leading to a decrease in evaporation from the soil. Moreover, the short duration of these two stages, which require only five irrigations, sharply contrast with the long vegetative growth stage; also, these two stages happen in December. At a time when temperatures are falling sharply. In general, the total seasonal water consumption was 262, 233, and 186 mm for the 25%, 40%, and 55% depletion levels of available water, respectively, under the drip irrigation system. This finding highlights the advantages of the drip irrigation system, as it gradually supplies water to the soil, maintaining a stable moisture level in the root zone. The slow application rate of water reduces evaporation losses, lateral seepage, and deep percolation, thereby enhancing irrigation water efficiency, promoting better plant growth, and minimizing weed growth.

Table 2. Effect of Moisture Depletion Level on Seasonal Water Consumption of Lettuce Under the Surface Drip Irrigation System

Depletion Level	Seasonal Water Consumption (mm)	Seedling Stage (days)	Vegetative Growth Stage (days)	Head Formation Stage (days)	Maturity Stage (days)	Total Duration (days)
25% of AW	Growth Stage Duration (days)	20	35	19	7	81
	Number of Irrigations	6	7	3	1	17
	Seasonal Water Consumption per Stage (mm)	98	81	58	25	262
	Daily Water Consumption (mm)	4.9	2.3	3.1	3.6	
40% of	Growth Stage Duration	20	35	19	7	81

AW	(days)					
	Number of Irrigations	6	7	3	1	17
	Seasonal Water					
	Consumption per Stage (mm)	98	68	48	19	233
	Daily Water Consumption (mm)	4.9	1.9	2.5	2.7	
	Growth Stage Duration (days)	20	35	19	7	81
55% of	Number of Irrigations	6	7	3	1	17
	Seasonal Water					
AW	Consumption per Stage (mm)	98	42	32	14	186
	Daily Water Consumption (mm)	4.9	1.2	1.7	2.0	

Table 3 presents the effect of moisture depletion levels (irrigation levels), proline foliar application, and their interactions on average head length (cm), head circumference (cm), and total yield ($\mu\text{g ha}^{-1}$). The average head length was 43.51, 39.12, and 33.90 cm at 25%, 40%, and 55% moisture depletion levels, respectively. The head circumference was 67.50, 61.15, and 51.51 cm, while the total vegetative yield of lettuce was 247.67, 215.10, and 171.73 $\mu\text{g ha}^{-1}$ at 25%, 40%, and 55% depletion levels, respectively. The irrigation level had a positive impact on increasing head length, head circumference, and lettuce yield, which can be attributed to the availability of adequate soil moisture and the absence of water stress during the growth stages. Maintaining 25% depletion of available water helped in retaining higher moisture content in lettuce leaf tissues and developing a well-established root system compared to the 40% and 55% depletion levels, which negatively affected root growth, expansion, and plant size, ultimately reducing growth parameters and total yield (6, 13, 18, 27). It is undeniable that low seasonal water consumption had negative effects on plant physiology and growth, leading to a decline in growth parameters and total yield. Low water levels caused a big drop in lettuce harvest. This shows that how well lettuce grows and how much it gives is strongly linked to how much water it gets.

A significant improvement in the height of lettuce plants was obtained with the increasing concentration of proline amino acid. Foliar applied proline at 80 mg L^{-1} resulted in increased plant height of the lettuce plant at all irrigation levels (moisture depletion levels) up to 45.13, 41.50, and 35.42 cm at 25%, 40%, and 55% depletion levels, respectively. This might be due to the reason that proline amino acid acts as a source of nitrogen in the process of plant growth; it accelerates the synthesis of proteins in the plant and consequently increases its growth and height (16, 24). Furthermore, increased concentration of proline significantly improved head circumference and total lettuce yield where head circumference was 70.10, 64.33, and 55.87 cm and total lettuce yield stood at 258.30, 228.50, and 181.40 $\mu\text{g ha}^{-1}$ for 25%, 40%, and 55% depletion levels, respectively. Greater head circumference and total yield with more proline can come from the good part of proline foliar use in controlling osmotic potential plus help with making proteins and giving energy for plant growth. Also, proline helps cells from damage due to oxygen, works as an enzyme keeper, and keeps cell structures which help with better plant growth, longer cell size, stomatal opening, and improved photosynthesis, making growth traits better and making total yield higher (9, 19, 22, 28, 30).

Table 3. Effects of water loss and foliar application of proline on head length (cm), head circumference (cm), and total vegetative yield ($\mu\text{g ha}^{-1}$)

Treatments	Average Head Length (cm)	Head Circumference (cm)	Total Vegetative Yield ($\mu\text{g ha}^{-1}$)
A1B0	42.17	65.15	233.1
A1B1	43.22	67.24	251.6
A1B2	45.13	70.10	258.3
Mean	43.51	67.50	247.67
A2B0	37.21	58.34	205.6
A2B1	38.65	60.78	211.2
A2B2	41.50	64.33	228.5
Mean	39.12	61.15	215.10
A3B0	32.18	47.21	161.2
A3B1	34.10	51.45	172.6
A3B2	35.42	55.87	181.4
Mean	33.90	51.51	171.73
LSD 0.05 Irrigation	1.12	2.63	21.22
LSD 0.05 proline acid	0.84	0.75	11.81
LSD 0.05 Irrigation *pr	1.67	3.22	28.69

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