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Study of the Effect of Irrigation Method and Spraying with Amino Acids on Some Growth Indicators and Biochemical Characteristics of Tobacco Plants (*Virginia variety*)

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

The experiment was implemented at the Stakhris Agricultural Research Station in Latakia during the two successive seasons of Agricultural 2021/2022, according to a split-randomized block design, with three replicates for each treatment. The research aimed to study the effect of irrigation method and spraying with amino acids on the growth and productivity of tobacco plants, the Virginia tobacco variety (Nicotiana tabacum var. Virginie vk51), where the morphological and physiological

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characteristics were studied (plant height (cm) and total leaf surface area (m2/plant), and the content of air-dried tobacco leaves of biochemical compounds (total protein, soluble sugars, and nicotine (%)). Using the drip irrigation method with the third level of amino acids (I1A2) gave the best results, as it was significantly superior to the rest of the treatments, as the plant height value in this treatment reached (226 cm), which was reflected positively on the total leaf surface area of the plant (3.52 m²). /plant), and the qualitative characteristics of dry tobacco leaf improved, as the percentage of total protein and nicotine decreased and the percentage of total soluble sugars increased. Based on the above, it can be suggested to use the drip irrigation method with the third level of amino acids, given their significant role in stimulating growth in the Virginia variety and improving the quality of the resulting tobacco.

Keywords: Drip irrigation system; quality of tobacco; amino acids; tobacco plant; Virginia variety.

1. INTRODUCTION

The fluctuation of the area of land planted with tobacco and also the productivity is related to a set of factors that affect tobacco cultivation and production. The irrigation method used to irrigate the crop is one of these factors, as tobacco is irrigated in several ways, including surface irrigation, spraying, and dripping, each of which has advantages and disadvantages, noting that the optimal irrigation method for each crop and region differs from others, as it is determined by many factors, including the physical properties of the soil (its mechanical composition and field capacity), as well as the water Availability, the type of plant grown, labor, mechanization, and costs [1].

Drip irrigation is a modern method for irrigating crops. This technology ensures that water is delivered to the plant in small quantities and at high frequency in points and in very limited areas of soil, This method is also a type of localized irrigation, in which water is delivered to the plant in the form of drops or drops over the desired area, which is the area where the root system spreads [2].

Tobacco is one of the most important industrial crops in the world. The importance of this crop locally is due to its important role in the country's internal and external trade and, its reliance on it as a source of foreign exchange, as it ranks third after cotton and sugar beets in terms of area. There are thousands of farmers, workers and engineers who work in the field of tobacco production, manufacturing and marketing, which indicates its great importance in the Syrian economy [3].

Tobacco is one of the most widely adaptable crops to soil, climate, and agricultural processes, including irrigation and fertilization. This crop

responds with high sensitivity to weather factors and soil-related conditions, and even a slight change in these previous environmental conditions can lead to a difference in productivity and the quality of the dry leaf crop [4].

Tobacco production (Virginia) is closely related to the amount of irrigation water supplied to the crop as well as the amount of rainfall during the growing seasons [5].

Humidity greatly affects the quantity and quality of the crop, and the dates of exposing the plant to drought play an important role in this regard, Exposing the plant to drought in the second stage of leads to a decrease in the storage of dry matter in the leaves, and excessive humidity causes a decrease in the weight of the leaf area unit due to the slow growth process and thus a decrease in the amount of dry matter. Choosing the appropriate irrigation method and program, along with other agricultural methods, is one of the important factors for obtaining ideal productivity [6].

Jones & Rasnake [7] explained that many factors affect the quality of the final tobacco crop, such as soil type, fertility, cultivation method, climate, service operations, and drying process conditions. The condition of the cultivation medium and the availability of nutritional requirements are among the most important factors affecting the production of high-quality dry leaves. The green weight and dry weight of tobacco leaves, the final yield, the degree of quality of dry leaves, and the chemical composition of the dry leaf are affected by the difference in nutrients present in the soil and the supply of the soil with the necessary elements, in addition to the difference in the climatic conditions of the region and the difference in soil quality [8].

It can be said that the chemical composition of different types of tobacco is subject to many dynamically complex and unstable interventions. If we know that about (5000 compounds) have become known and precisely defined and enter into the composition of the dry tobacco leaf, and contribute to the release of the final flavors and tastes through complex mechanisms, we explore the importance and depth of the different and overlapping effects of many factors on this mentioned composition, including (the genetic factor, agriculture and various agricultural treatments, soil quality, fertilizer quality, climate conditions, plant diseases, the location of the leaves on the stem, harvest date, the drying process and its conditions, and the storage process.

However, it can be said in general that extreme proportions of protein, nicotine, nitrogenous compounds and chlorine negatively affect the quality of flavour and taste of the produced tobacco. On the other hand, high proportions of sugars (total soluble or reducing) positively affect the quality of flavours of the produced tobacco [9]. The changes that occurred in the chemical composition of the Virginia leaf when it was dried in ovens indicate that the percentage of starch reached (5.52%), the percentage of total sugars reached (23.77%), the percentage of total nitrogen was (1.05%), the percentage of protein nitrogen was (0.51%), and the percentage of nicotine was (0.97%) [8]. Fertilization treatments, soil quality, soil moisture, water quality, and irrigation method all have a decisive effect on the chemical composition and physical properties of the dry leaf.

In developing countries, farmers use large amounts of chemical fertilizers and pesticides to achieve high crop yields. Mineral fertilizer provides readily available nutrients to the plant and is often associated with excessive uptake of nitrates and sulphates that cause health problems for humans [10]. The addition of chemical fertilizers also has a harmful effect on the environment [11].

Therefore, recent attention has been paid to reducing sources of pollution from modern agriculture. One of the methods of reducing this pollution is the use of biostimulants, which have become widely used as a safe form of plant growth regulators, polyamines, and vitamins.

Biostimulants aim to reduce the impact of unfavorable environmental stresses on crops and stimulate their growth and development and enhance the size and quality of the crop [12]. Spraying with amino acids helps overcome nutrient deficiencies that occur during growth [13].

In recent years, the importance of relying on organic plant fertilizers (Bio-stimulants) has emerged, including amino acids, due to their role as safe growth regulators and as a clean natural source to reduce sources of pollution in agriculture, in addition to their role in increasing plant productivity [14].

Amino acids are the major form of organic nitrogen, readily transported in the incomplete and complete sap to all parts of the plant. They are either directly metabolized or used to synthesize proteins and stored in target tissues. Amino acids, as organic activators, rapidly affect the enzymatic activity in the plant, which leads to positive effects on plant growth and productivity and reduces damage caused by environmental and biotic stresses [15].

Previous studies have indicated that treatment with amino acids improved plant growth indicators and consequently productivity and quality in many crop plants, including: soybean [16], and strawberry [17].

2. THE IMPORTANCE OF THE RESEARCH AND ITS OBJECTIVES

2.1 Importance of Research

Despite the great importance of water resources in Syria and the limited nature of these resources, the efficiency of their use in the agricultural sector is still low and does not exceed 40-50% in the best cases. It can be said that improving the efficiency of agricultural irrigation by about 10% will save a large amount of dam water, considering that agriculture consumes about 86% of the total invested resources.

Nfluctuations and instability due to the lack of integration of available water resources. The exploited water is exposed to waste and loss due to the low efficiency of irrigation networks and the use of old traditional irrigation methods. Many studies have proven that amino acids play a positive role in enhancing plant growth and quality when sprayed at different growth stages, especially critical ones such as the tillage and flowering stages or under stress conditions (such as drought, high temperature, frost, salinity or pathogens), Therefore, its importance and effectiveness lie in the stages of plant growth, as

it contributes to increasing the cell's ability to absorb water and nutrients dissolved in the growth medium, which is positively reflected in vegetative growth and thus increasing the crop and improving quality.

2.2 Research Objective

Study of the effect of irrigation method with amino acid spraying on the growth and productivity of tobacco plant (Virginia variety).

3. MATERIALS AND METHODS

3.1 Study Site

The research was conducted in two agricultural seasons (2021/2022) at the Stakhris Agricultural Research Station in Lattakia, Syria (about 17 km away from Lattakia Governorate) at latitude 35.52 and longitude 35.33, and 13 m above sea level. The study area is characterized by hot summers with cold and rainy winters with two transitional seasons characterized by moderate and unstable weather.

3.2 Study Site Soil

This research was conducted on a soil with a loamy texture and a pH that tends to be alkaline, medium content of organic matter, abundant in mineral nitrogen and good content of available phosphorus and potassium, rich in total calcium carbonate and it is a natural soil according to the classification of the scientist, Some physical and chemical analyses of the site soil were conducted on samples taken before planting in the laboratories of the Faculty of Agriculture - Tishreen University, and the results of the analysis came as shown in Table 1.

3.3 Parameters Used and Experimental Design

The research used Virginia tobacco (Kotsaka VK51) (Nicotiana tabacum var. Virginie vk51) seedlings ready for planting in permanent ground. The experiment was designed according to split randomized blocks, where the number of

treatments was (6) and the number of replicates for each treatment was (3) thus the number of experimental plots was (18 The plant density was 2.5 seedlings/m² (the distance between the lines was 90 cm and between the seedlings was 40 cm) and the area of the experimental plot was 8 m² and the width of the aisles was 1.5 m², the total area of the experiment was 144 m². The two irrigation method treatments and three levels of spraying with amino acids were arranged as follows

Titel method treatments:

- 1- Rest irrigation: (I2), plunger flow 1 L/s (operating time 1 hour, so that each experimental plot was irrigated at a rate of 180 L/h
- 2- Drip irrigation: (I1), drip discharge 6 L/h, operating time 1 hour.

The plants were startedirrigated at transplantation in addition to rigation after 10 days transplantation, noting that the irrigation rate varies according to the growth stage and soil type, in addition to the prevailing environmental weather factors). Irrigation was stopped when the middle leaves matured in order to accelerate maturity and obtain the appropriate technological specifications, and harvesting was done 12 days after the last irrigation.

Fertilizers were added as solid mineral fertilizers (nitrogenous, phosphorous): ammonium nitrate (33.5%) and triple superphosphate (48%), and as soluble mineral fertilizers (potassium): potassium sulphate (50%), Phosphorous and potassium fertilizers were added at once with basic tillage of the land, and seeding was done on 6/5/2021, and nitrogen fertilizers were added in two batches: the first batch 10 days after seeding, and the second 25 days after the seeding date. The experiment was irrigated during the growing season 9 times in addition to the two irrigations (seedling and cooling). Agricultural service operations were carried out from controlling (herbicides, insecticides and fungicides).

Table 1. Some physical and chemical properties of the experimental soil before planting

SAR	O.M.%	CaCo ₃ Active %	CaCo ₃ Total%	EC Milmus/cm	PH	Texture	Sand%	Silt%	Clay %
0.932	1.43	15.8	49.7	0.198	7.8	Loamy clay	40	17	43
ESP %	Total porosity	True density	Bulk density	N	K	P (mg/kg) dr	Mg y soil	Ca	Na
0.317	<u>%</u> 53.7	g/cm³ 2.57	g/cm³ 1.19	10	182	18	516	1940	266

Table 2. Experimental diagram

I1A0	I1A1	I1A2	
I1A2	I1A0	I1A1	
I1A1	I1A2	I1A0	
I2A1	I2A0	I2A2	
I2A0	I2A1	I2A2	
12A2	I2A0	I2A1	

3.4 Experimental Design

The experiment was designed according to the split-block system, and the results were statistically analyzed using the CoStat statistical analysis program and the ANOVA test and calculating the least significant difference (LSD) at a significance level of 0.05% to determine the significant differences between the means.

The following properties and characteristics were studied:

- 1- Formal readings: Plant Height (cm/plant): 10 plants are randomly selected from each experimental plot to determine the height, where the plant height (cm) is determined starting from the soil surface level, as the plant enters the flowering stage (two months after transplanting.
- **2- Plant Leaf Area (cm2):** The total plant leaf area (PLA) was calculated as follows;

Paper area (cm2) = Paper length (cm) \times Paper width (cm) \times 0.6443 [18].

Total leaf area (cm2/plant) = sum of the area of all leaves of the plant.

3-Biochemical indicators: Leaf samples were taken from three plants (n=3) for each experimental treatment at maturity to measure the following biochemical parameters.

3.4.1 Total protein content

Proteins and total nitrogen were determined by the Kjeldahl method, assuming that proteins contain one sixth of their weight in nitrogen. The protein was digested by prolonged boiling with 98% concentrated sulfuric acid, converting the nitrogen of amino acids to sulfate. Ammonium. After digestion was complete, distillation was carried out to remove ammonia from the ammonium sulfate by adding sodium hydroxide NaOH and heating, where the ammonia combines with boric acid to form ammonium borate.

Ammonium borate was titrated as a final step by standard HCl with a suitable indicator to determine the end point of the titration [19].

3.4.2 Total soluble sugar content

Sugars were extracted from the leaf samples by boiling them with water, then centrifuging and filtering. The extracted total sugars were reacted with sulfuric acid to release furfural from the pentose sugars, and hydroxymethylfurfural from the hexose sugars, and then reacted with the organic reagent (anthrone) to form a blue-green color whose intensity is proportional to the concentration of sugars in the tested samples. The optical absorption of the formed colors was measured using a (Spectrophotometer) device at a wavelength of (620) nm, and the concentration of sugars was estimated through a standard curve that was created using standard solutions [20].

3.4.3 Nicotine content

Tobacco alkaloids were extracted by a mixture of benzene and chloroform in the presence of barium hydroxide, and nicotine in the extract was determined by standard acid, perchloric acid [21].

4. RESULTS AND DISCUSSION

4.1 Effect of Irrigation Method with Amino Acid Spraying on Tobacco Plant Height (cm)

The results shown in Fig. 1 regarding the effect of irrigation method with amino acid spraying on tobacco plant height indicate the emergence of significant differences between the studied Treatment (I1A2) significantly outperformed the rest of the treatments. Statistical analysis shows the existence of significant differences after calculating the (LSD) value for the least significant difference between the treatments (LSD=2.684). Given the importance of amino acids as an organic stimulant, as they can be quickly absorbed and transported within the different parts of the plant, due to their direct effect on the enzymatic activity of the plant, in addition to their involvement in the composition and synthesis of growth hormones, which explains this tangible positive effect of spraying amino acids at concentrations of 5 and 10 g/L on some growth characteristics that were measured. In this context, Abu Dahi and Al-Younis [22] indicated that spraying amino acids on the plant's vegetative system improves the plant cell's ability to draw water and dissolved nutrients from the growth medium, thus increasing vegetative growth, and also contributes to the length of the period of cell divisions and their number, in addition to the growth and development of dividing, Fig. 1 also, reveals that the irrigation method affects plant height, and the tallest plants were obtained under drip irrigation with all spraying amino acid treatments.

The continuous availability of water, even in small quantities, in the root zone, as a result of the shorter interval of irrigation periods under drip irrigation, led to absorb more water and nutrients by the plant, which led to the elongation of the stems and shoots of the plant.

4.2 The Effect of Irrigation Method with Amino Acid Spraying on the Total Leaf Surface Area of the Plant (m²/plant)

The results of the statistical analysis showed that there were statistically significant differences after calculating the (LSD) value for the least significant difference between the treatments regarding the total leaf area of the plant (LSD = 0.150), and this result is consistent with what the researchers reached [23], as the drip irrigation method contributed to increasing the leaf area of the plant, which positively reflects on the plant's productivity. Many studies have shown that spraying plants with organic fertilizers that contain amino acids in their composition led to accelerating their growth, increasing their leaf area and leaf chlorophyll content, in addition to

increasing production and improving its quality [24].

4.3 The Effect of Irrigation Method with Spraying Amino Acids on the Percentage of Nicotine (%)

It is noted from Fig. 3 that there is a decrease in statistically significant differences, especially between the two irrigation methods (rest-drip) (LSD=0.134).

The drip irrigation method contributed to securing water better around the roots, which contributed to reducing the percentage of nicotine in the leaves, noting that the limit permitted by the General Tobacco Corporation for the Virginia variety regarding nicotine is (2-1.5%).

It was also found that the percentages of nicotine (since nicotine is one of the most important nitrogenous bases found in dry tobacco leaves, if not the most important of all) decrease clearly as the percentage of moisture in the soil increases, which is positively reflected in the quality of dry leaves [9].

Sullivan et al. [25] indicates that the roots are the main factory of nicotine and most of the nicotine produced in the roots is not retained, but rather moves to other parts, especially the leaves, and accumulates there. Iami and Honarnejad [26] confirm that although nicotine levels are largely subject to genetic factors, they are, in contrast, highly affected by environmental factors and agricultural processes.

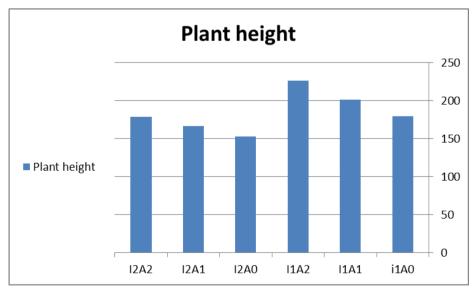


Fig. 1. Effect of irrigation method with amino acid spraying on tobacco plant height (cm)

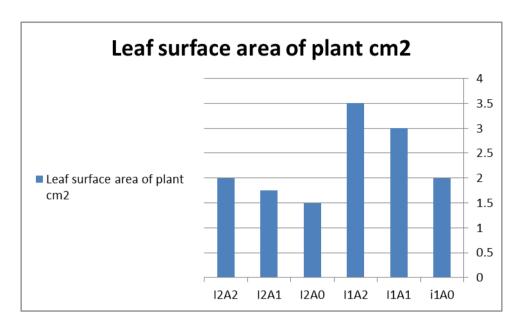


Fig. 2. The effect of the irrigation method with amino acid spraying on the total leaf surface area of the plant (m²/plant)

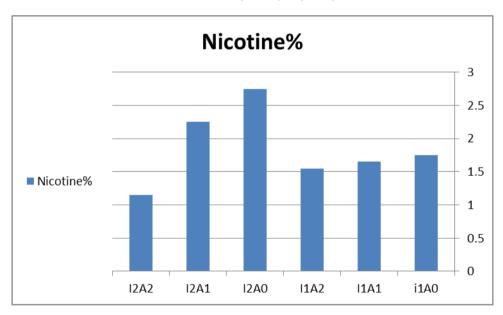


Fig. 3. The effect of irrigation method with spraying with amino acids on the percentage of nicotine (%)

Spraying contributed to with amino acids reducing the content of nicotine and proteins in the leaves. This effect may be due to the role of the amino derivative glycine betaine GB, due to its role in stimulating plant growth and improving the vital towards metabolic cycles the plant in increasing and regulating the secretion of growth hormones on the one hand and increasing the composition of soluble sugars, which leads to a decrease in the rate of synthesis and accumulation of nicotine and proteins in the plant.

4.4 Effect of Irrigation Method with Amino Acid Spraying on Protein Percentage (%)

It is noted from Fig. 4 that the percentage of protein recorded a clear decrease affected by the irrigation method, as the decrease in the percentage of protein was statistically significant

(LSD = 0.279), and with clear significant differences between the studied treatments, noting that the limit permitted by the General Tobacco Corporation for the Virginia variety regarding protein is (7-5.5%).

Studies on the percentages of protein within the dry tobacco leaf also indicate that the higher the soil moisture, the lower the ability of the leaves to manufacture protein, as nitrogenous materials negatively affect the quality of tobacco, with the exception of nicotine within certain limits. Protein is one of the most negative nitrogenous compounds on the quality of tobacco, because protein gives off an unpleasant odor when tobacco is burned, similar to the smell of burning feathers, as the main product of protein combustion is ammonia [27].

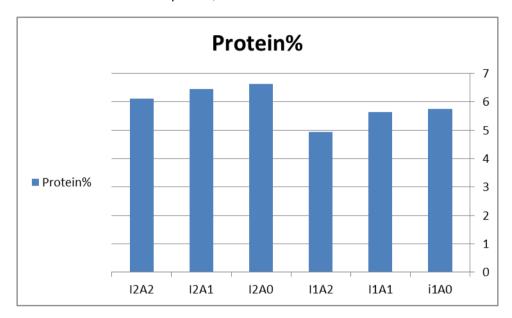


Fig. 4. The effect of irrigation method with amino acid spraying on the protein percentage (%)

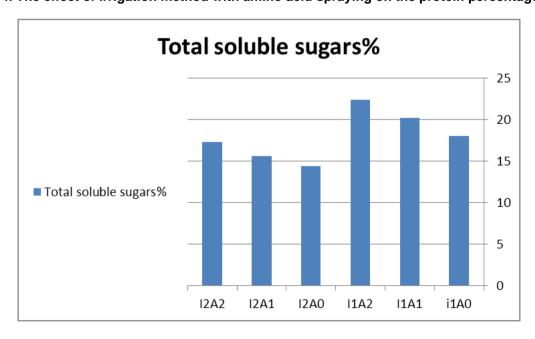


Fig. 5. Effect of irrigation method with amino acid spraying on the percentage of total soluble sugars (%)

4.5 Effect of Irrigation Method with Amino Acid Spraying on the Percentage of Total Soluble Sugars (%)

These specific compounds (reducing and nonreducing sugars) were affected by irrigation methods and levels of amino acid spraying, where the drip irrigation method with the third level of amino acids (10 g/L) had the greatest effect on the accumulation of these specific and very important compounds in the Virginia variety, and significant statistical differences were recorded between the studied treatments (LSD = 0.071). Most research and studies have shown that in very humid lands, plant growth intensifies, enabling it to synthesize larger quantities of carbohydrates at the expense of nitrogenous compounds [28]. Sugars are an indicator of quality in tobacco because they have the greatest effect on the specifications of the dry leaf, both taste and technology, because when they accumulate within dry tobacco leaves, the overall characteristics of flavor, taste and physical properties of the leaf improve significantly [9].

Note that the permissible limits for total sugars for the Virginia variety according to the General Tobacco Organization are (29.09-7.86%).

This increase in the percentage of total soluble sugars at concentrations of 5 and 10 g/L may be due to the effect of amino acids and their role in activating all the physiological, biochemical metabolic processes taking place in the cells, in addition to providing ready-made components for building, assembling and synthesizing sugars, which is positively reflected in the quality of Virginia tobacco [29].

5. CONCLUSIONS AND RECOMMENDA-TIONS

5.1 Conclusions

The results showed that the Virginia tobacco variety responded positively to the drip irrigation method with the third level of spraying with amino acids, which appeared in most of the studied morphological, physiological, productive and biochemical characteristics and traits, as the use of treatment (I1A2) led to giving the best results regarding the morphological indicators of the tobacco plant (plant height - total leaf surface area), as the drip irrigation method with spraying with amino acids contributed to improving the

qualitative specifications of dry tobacco leaves, as the percentage of total protein and nicotine decreased and the percentage of total soluble sugars increased.

5.2 Recommendations

Based on the research results, we recommend the following: Using drip irrigation method with the third level of amino acids 10 g/l, due to its tangible role in stimulating growth in the Virginia variety and improving the quality of the resulting tobacco. It is preferable to continue the research for several years to know the effect of irrigation methods with spraying with amino acids to include more morphological and technological characteristics of the tobacco plant.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that generative Al technologies such as Large Language Models, etc have been used during writing or editing of this manuscript. This explanation will include the name, version, model, and source of the generative Al technology and as well as all input prompts provided to the generative Al technology.

Details of the Al usage are given below:

- 1. Al technologies analyze and use data to help business processes more effectively.
- 2. Developers are using AI to perform tasks that are done manually more efficiently.
- 3. Artificial intelligence is the technology that enables machines to exhibit human-like reasoning and capabilities such as autonomous decision-making.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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