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## **The Effect of Green Manure and Irrigation on Morphological and Physiological Characteristics of Virginia (Flue-Cured) Organic Tobacco (*Nicotiana tabacum*)**

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**Abstract:** Field experiments were conducted to determine the effects of drip irrigation and sprinkler irrigation and green manure on morphological and physiological characteristics of tobacco. The experiment was laid out in a split plot design with four replicates, two main plots (drip and sprinkler irrigation) and three sub-plots (vetch as green manure, red clover as green manure and control (without fertilization)). Green manures increased leaves width and length, diameter of stem and roots and the most significant impact was when vetch was applied to soil. Physiological characteristics were significantly influenced by the green manures. The lowest stomatal conductance and photosynthetic rate were found in control plots. Drip irrigation was characterised by a smaller amount of water applied to the soil. However, drip irrigation increased the length of leaves. The roots and stem diameter were not affected by the irrigation systems. Also, 86 days after transplanting, the lowest stomatal conductance and photosynthetic rate were found in plots irrigated with sprinkler system.

**Key words:** Irrigation system, tobacco, transpiration rate, chlorophyll, photosynthetic rate, stomatal conductance

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### **INTRODUCTION**

The increasing world-wide shortages of water and costs of irrigation are leading to an emphasis on developing methods of irrigation that minimize water use (maximize the water use efficiency). Irrigation is critical for successful summer plant production in Greece. Drip irrigation has not been widely used in tobacco production, because of the higher cost of the drip line installation. In the recent years, the cost of the installation has relatively decreased, because of the technology improving. Advanced of drip irrigation systems as compared to the overhead irrigation systems includes reduced water use (Cetin and Bilgel, 2002; Sharmasarkar *et al.*, 2001) and decreased weed growth (Sabra, 2000; Veeraputhiran and Kandasamy, 2001). Also, other investigators have reported high yields for crops under drip irrigation (Cetin and Bilgel, 2002; Malash *et al.*, 2005; Tiwari *et al.*, 2003; Yohannes and Tadesse, 1998).

Soil moisture availability is the main factor influencing nitrogen uptake by the root and transport to the leaf. Sifola and Postiglione (2003) reported that nitrogen uptake rate was increased by either irrigation or nitrogen fertilization. Despite the fact that tobacco Virginia type is usually irrigated and fertilized, little work has been done on the combined effect of irrigation and nitrogen fertilization on growth of tobacco.

Green manuring is the process of turning a crop into the soil. Green manures are known to increase soil N, P and K availability for the following crop and at the same time, contribute to the conservation of soil organic matter and soil biological, physical and chemical properties

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(Huxham *et al.*, 2005; Mandal *et al.*, 2003; Sangakkara *et al.*, 2004). Data obtained by others researchers (Arshad and Gill, 1997; Aulakh and Pasricha, 1998; Becker *et al.*, 1990; Bilalis *et al.*, 2005; Danso and Papastylianou, 1992; Mohammmand and Mahmood, 1997; Pal and Shedu, 2001; Sangakkara *et al.*, 2004; Stopes *et al.*, 1996) clearly demonstrated the beneficial effects of legumes on growth of the following crops (wheat, barley, canola, maize, mustard, rice).

The specific objectives of the present study were to determine the effects of irrigation system and green manure on morphological and physiological characteristic of tobacco plants.

## MATERIALS AND METHODS

### Experimental Design

The experiment was repeated twice. A tobacco crop (*Nicotiana tabaccum* cv. NC71) was established in the experimental organic field of the Agricultural University of Athens (23.43E, 34.58N). The soil was clayloam (29.8% clay, 34.3% silt and 35.9% sand) with pH 7.24, 1.17% organic matter and 0.54 mS cm<sup>-1</sup> of EC. In parallel, a tobacco crop was established in the Domokos (Central Greece) district (22.33E, 39.03N) 200 km north of Athens. The soil was clay (50.7% clay, 23.3% silt and 26% sand) with pH 7.6, 2.2% organic matter and 0.32 mS cm<sup>-1</sup> of EC. The crops cultivated before tobacco were vetch (*Vicia sativa* cv. Alexandros) and red clover (*Trifolium pratense* cv. Nemaro) which was incorporated into the soil, during the flowering stage. Prior to this study the field was under wheat cultivation.

The experiment was set up on an area of 1200 m<sup>2</sup> according to the split plot design with four replicates, two main plots (irrigation systems) and the tree sub-plots (fertilization treatments). The fertilization treatments were: Vetch and red clover as green manures and control plots (without fertilization). The plot size of each irrigation systems was 15×10 m and the sub-plot size was 5×10 m.

### Irrigation Systems

Overhead sprinkler and drip irrigation systems (Table 1) were set up on adjacent blocks within the same field, but enough far in order to avoid cross irrigation. Soil moisture content was measured with capacitance soil moisture Probe (Didcot. Instrument, Co Ltd. Abingdon, Oxfordshire, England). The amounts and dates of irrigation were determined in the light of such measurements. Irrigation occurred when the available soil moisture reached 70% and sufficient water was added to return the soil moisture to field capacity.

The drip system consisted of PE laterals with 20 mm diameter. The laterals have in line drippers and at 0.40 cm distance. The drippers had a discharge rate of 4 L h<sup>-1</sup> under an operation pressure of 1 atm.

The sprinkler irrigation systems consisted mainly pipes of 40 mm diameter, laterals pipes of 40 mm diameter and rotor type sprinklers (radius: 5 m, discharge rate: 16.35 L min<sup>-1</sup>) with 2 m risers. Laterals pipes were 15 m long with sprinklers spaced at 5 m in each lateral.

Table 1: Total water requirements (quantity of water applied (mm) and number of irrigations) on the experimental sites

Irrigation system	Area	Total Quantity of water applied (mm)	No. of irrigation
Drip irrigation	Domokos	200	8
	Athens	252	10
Sprinkler irrigation	Domokos	350	8
	Athens	397	10

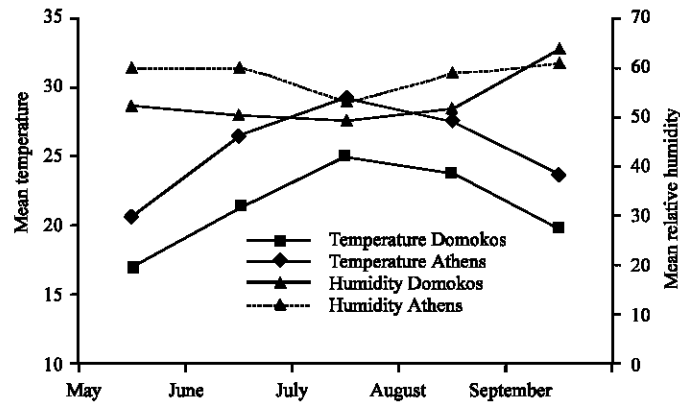


Fig. 1: Monthly mean temperatures and relative humidity during the growing season for tobacco (May-September)

#### Planting, Fertilization and Weed Control

Vetch and red clover were sown by hand in rows of 20 cm apart at a depth of 1.5 cm. The field in Domokos district, was sown on 1 October 2004 and in Athens, was sown on 4 November 2004 at a rate of 100 and 20 kg ha<sup>-1</sup> for vetch and red clover, respectively. Vetch and red clover was incorporated into the soil at the beginning of May.

Seeds of tobacco were sown on the 5th of March 2005 in a seedbed under plastic house conditions (to protect seedling from cold weather). After hardening, seeding transplanting on the 18th and 22nd May, in the experimental field of the Agricultural University of Athens and in Domokos district, respectively. Seedling was transplanted by hand in rows of 90 cm apart. Transplants were set at 30 cm between each other. Also, the weeds were controlled by hand. Some climatological data on the experimental sites are presented (Fig. 1).

#### Sampling, Measurements and Methods

##### Legumes

Biomass samples were taken at the end of April. To define biomass the plants were cut at 1 m in 2 different places of each plot. The total N from biomass were measured with the Kjeldahl method (Bremer, 1960).

##### Tobacco

##### Length, Width of Leaves and Diameter of Stem

The sampling were made 114 days after the transplanting date of tobacco. Width of leaves, length of leaves (the fifth leaf of the plant) and diameter of stem (at the base of plant) were determined by measurement of 5 plants per plot.

##### Roots

Root samples were taken 114 days after the transplanting date of tobacco. Samples were collected from each plot at soil depth 0-30 cm with a 30×30 cm sampler. Each sample roots were separated from soil after standing for 24 h in water + (NaPO<sub>3</sub>)<sub>6</sub> + Na<sub>2</sub>CO<sub>3</sub>. For the determination of the diameter of roots, the root samples were placed on a high-resolution scanner using DT-software (Delta-T Scan version 2.04; Delta-T Devices Ltd, Burrwell, Cambridge, UK).

### Physiological Characteristics

During growth there is a period (41-75 days after transplanting) when dry matter and nutrient uptake in plants occurs at an intense rate (Moustakas and Ntzanis, 2005). The physiological characteristics were determined: 47 days after transplanting (DAT), 62 DAT and 86 DAT.

### Stomatal Conductance, Photosynthetic Rate and Transpiration Rate

Stomatal conductance, photosynthetic rate and transpiration rate were determined using a Lci Leaf Chamber Analysis System (Lci). This instrument comprises a main console with signal conditioning, air sample and a leaf chamber. The main console supplies air with a relatively stable CO<sub>2</sub> concentration to the chamber at a measured rate. The CO<sub>2</sub> and H<sub>2</sub>O concentrations are measured and the air is directed over both surfaces of the leaf. The discharged air leaving the chamber is analysed and its CO<sub>2</sub> content and H<sub>2</sub>O content determined. From the differences in gas concentration and the airflow rate, the stomatal conductance, photosynthetic rates and transpiration rates, are calculated approximately every 20 sec.

### Statistical Analysis

Statistical analyses such as variance and mean comparisons were carried out using Statistica (Statsoft Inc., Tulsa, OK).

## RESULTS AND DISCUSSION

### Green Manure

The application of Vetch and Red clover increased the rate of root thickening. The highest roots diameter (Table 2) was found when vetch was applied to soil. This species which accumulated the highest total N (Table 3) stimulates tobacco growth to a greater extent than red clover. Data obtained by others researchers (Mandal *et al.*, 2003; Sangakkara *et al.*, 2004; Stopes *et al.*, 1996) clearly demonstrated the beneficial effects of legumes on the root growth of the following crops (wheat, maize, rice). The favourable effect of these treatments on root growth was likely due to the increase in organic

Table 2: Influence of irrigation system and fertilization on diameter of tobacco roots (mm)

Fertilization	Athens		Domokos	
	Irrigation system		Irrigation system	
	D	S	D	S
<b>Diameter of roots</b>				
Vetch	0.65	0.67	0.98	1.36
Control	0.61	0.62	0.69	0.77
Red clover	0.72	0.66	0.81	0.91
LSD <sub>irrigation</sub> (p = 0.05)		0.30		0.42
LSD <sub>fertilization</sub> (p = 0.05)		0.09		0.28

D: Drip irrigation; S: Sprinkler irrigation

Table 3: Amount of N (kg ha<sup>-1</sup>) that accumulated in the roots and in above-ground parts (multiplying Dry Weight kg ha<sup>-1</sup> with N%) of the vetch and red clover plants (Mean values ± Standard Error)

Amount of N in different plant parts	Domokos	Athens
N roots-Vetch	76.5±2.80	77.4±2.00
N biomass-Vetch	170.8±12.4	157.9±21.2
N total-Vetch	247.3±12.6	235.3±22.6
N roots-Red clover	82.8±2.60	71.6±3.00
N biomass-Red clover	78.4±6.50	41.0±8.40
N total-Red clover	161.2±5.20	112.6±8.80

matter leading to better physical (bulk density and soil aggregation) and chemical conditions (total soil nitrogen) (Mandal *et al.*, 2003). Poor soil physical conditions were mainly response for the restricted root growth. The highest length, width of leaves and diameter of stem was obtained with the incorporation of vetch followed by red clover. Also, Pramanik *et al.* (2004) clearly demonstrated the beneficial effects of legumes on growth of transplant Aman rice. The highest grain yield of rice was obtained with the incorporation of *Sesbania rostrata* followed by *Sesbania aculeata*. Also, the lowest grain yield was obtained with the incorporation of *Crotalaria juncea* probably due to less amount of biomass added during incorporation.

Moreover, physiological characteristics were significantly influenced by the green manures. Nitrogen is the main constituent of proteins, chlorophyll and enzymes involved in photosynthesis. Therefore, nitrogen affect photosynthesis of crops. The lowest stomatal conductance (Table 4) and photosynthetic rate (Table 5) were found in control plots. In general, the lowest transpiration rate was also found in control plots (Table 6). Selvaraju and Iruthayaraj (1995) reported that stomatal conductance and transpiration rate of maize plants were increased with N application. Moreover, other investigators have reported high stomatal conductance, transpiration and photosynthetic rates for plants (sunflowers, maize and tomato) under nitrogen application (Cechin, 1998; Cechin and Terezinha, 2004; Guidi *et al.*, 1998). The reduced photosynthetic rate resulted in a significant reduction in growth of tobacco plants. Thus, the highest correlation coefficient ( $r = 0.88^{***}$ , Table 7) between photosynthetic rate and width of leaves was found in Athens region. Also, the highest correlation coefficient ( $r=0.88^{***}$ , Table 7) between photosynthetic rate and length of leaves was found in Athens region. Sifola and Postiglione (2003) reported that both dry matter accumulation and yield of leaves were increased by nitrogen fertilization. Also, Angellino *et al.* (2004) and Sifola *et al.* (2003) reported that nitrogen fertilizer application positively influenced the leaf area of tobacco plants.

### Irrigation System

Drip irrigation was characterised by a smaller amount of water applied to the soil (Table 1). In this study, we used 40% less water with drip irrigation as compared to sprinkler irrigation. However, the tobacco growth was not affected by the reduced water application. Considering the nature of drip

Table 4: Influence of irrigation system and fertilization on stomatal conductance ( $\text{mol m}^{-2} \text{sec}^{-1}$ ) (47, 62 and 86 days after transplanting: DAT)

Fertilization	Athens		Domokos	
	Irrigation system		Irrigation system	
	D	S	D	S
<b>47 DAT</b>				
Vetch	0.37	0.21	1.32	1.60
Control	0.35	0.19	0.85	0.78
Red clover	0.31	0.21	1.03	1.18
LSD <sub>irrigation</sub> ( $p = 0.05$ )		0.03		0.15
LSD <sub>fertilization</sub> ( $p = 0.05$ )		0.02		0.12
<b>62 DAT</b>				
Vetch	0.22	0.06	1.22	0.64
Control	0.07	0.02	0.78	0.21
Red clover	0.30	0.11	1.28	0.53
LSD <sub>irrigation</sub> ( $p = 0.05$ )		0.03		0.11
LSD <sub>fertilization</sub> ( $p = 0.05$ )		0.02		0.07
<b>86 DAT</b>				
Vetch	0.61	0.46	0.25	0.20
Control	0.08	0.07	0.09	0.05
Red clover	0.81	0.21	0.16	0.11
LSD <sub>irrigation</sub> ( $p = 0.05$ )		0.05		0.02
LSD <sub>fertilization</sub> ( $p = 0.05$ )		0.05		0.01

D: Drip irrigation; S: Sprinkler irrigation

Table 5: Influence of irrigation system and fertilization on photosynthetic rate ( $\mu\text{mol m}^{-2} \text{sec}^{-1}$ ) (47, 62, 86 days after transplanting: DAT)

Fertilization	Athens		Domokos	
	Irrigation system		Irrigation system	
	D	S	D	S
<b>47 DAT</b>				
Vetch	5.70	1.86	21.99	21.67
Control	3.03	1.46	17.41	19.65
Red clover	3.66	1.79	19.34	20.28
LSD <sub>irrigation</sub> (p = 0.05)		0.34		0.36
LSD <sub>fertilization</sub> (p = 0.05)		0.19		0.70
<b>62 DAT</b>				
Vetch	12.46	9.84	21.67	17.69
Control	6.89	2.05	16.59	11.79
Red clover	11.09	7.97	20.50	16.30
LSD <sub>irrigation</sub> (p = 0.05)		0.76		0.61
LSD <sub>fertilization</sub> (p = 0.05)		0.37		0.84
<b>86 DAT</b>				
Vetch	18.30	15.93	11.6	10.57
Control	6.36	5.97	6.28	3.39
Red clover	16.15	12.42	9.36	9.01
LSD <sub>irrigation</sub> (p = 0.05)		0.33		0.25
LSD <sub>fertilization</sub> (p = 0.05)		0.63		0.44

D: Drip irrigation; S: Sprinkler irrigation

Table 6: Influence of irrigation system and fertilization on transpiration rate ( $\text{mmol m}^{-2} \text{sec}^{-1}$ )(47, 62, 86 days after transplanting: DAT)

Fertilization	Athens		Domokos	
	Irrigation system		Irrigation system	
	D	S	D	S
<b>47 DAT</b>				
Vetch	4.56	4.32	12.84	13.54
Control	4.46	4.27	12.87	12.35
Red clover	5.14	4.50	12.47	12.20
LSD <sub>irrigation</sub> (p = 0.05)		0.16		0.72
LSD <sub>fertilization</sub> (p = 0.05)		0.20		0.40
<b>62 DAT</b>				
Vetch	6.43	3.34	10.62	10.08
Control	1.94	0.58	9.50	6.55
Red clover	5.82	3.44	10.80	10.69
LSD <sub>irrigation</sub> (p = 0.05)		0.31		0.41
LSD <sub>fertilization</sub> (p = 0.05)		0.19		0.43
<b>86 DAT</b>				
Vetch	8.58	7.53	6.15	3.49
Control	3.04	3.48	4.09	2.30
Red clover	7.52	5.59	6.14	2.69
LSD <sub>irrigation</sub> (p = 0.05)		0.25		0.36
LSD <sub>fertilization</sub> (p = 0.05)		0.17		0.26

D: Drip irrigation; S: Sprinkler irrigation

irrigation, where irrigation water is applied slowly and frequently, the soil water content in a portion of the plant root zone remains fairly constant compared to other irrigation methods (Cetin and Bilgel, 2002). Soil moisture availability is the main factor influencing nitrogen uptake by the root and transport to the leaf. The roots and stem diameter were not affected by the irrigation systems. Drip irrigation increased the length of leaves (Table 8). The possible cause of this may be that weed density in sprinkler irrigation was higher as compared to drip irrigation. In our study, weed density, in particular *Orobancha ramosa* (Karkanis *et al.*, 2007) and *Amaranthus retroflexus* was more in sprinkler

Table 7: Correlation coefficients<sup>1</sup> between plant parameters of tobacco

Plant parameters	Athens	Domokos
Stomatal conductance × photosynthetic rate	0.88***	0.95***
Stomatal conductance × transpiration rate	0.88***	ns
Transpiration rate × photosynthetic rate	0.99***	0.51*
Width of leaves × photosynthetic rate	0.88***	0.64**
Length of leaves × photosynthetic rate	0.88***	0.63**

1: r was calculated using the linear equation, ns: no significant. \*, \*\*, \*\*\*: Significant at p = 0.05, 0.01 and 0.001, respectively

Table 8: Influence of irrigation system and fertilization on length of leaves (cm), width of leaves (cm) and diameter of stem (cm) of tobacco plants (114 days after transplanting: DAT)

Fertilization	Athens		Domokos	
	Irrigation system		Irrigation system	
	D	S	D	S
<b>Length of leaves</b>				
Vetch	59.62	53.00	50.18	48.12
Control	44.96	40.40	42.43	41.81
Red clover	51.31	48.65	47.18	47.00
LSD <sub>irrigation</sub> (p = 0.05)		2.66		0.69
LSD <sub>fertilization</sub> (p = 0.05)		2.55		2.87
<b>Width of leaves</b>				
Vetch	28.84	26.40	25.12	26.50
Control	19.60	18.02	21.43	21.31
Red clover	24.31	23.05	23.06	23.50
LSD <sub>irrigation</sub> (p = 0.05)		0.93		2.47
LSD <sub>fertilization</sub> (p = 0.05)		1.72		1.15
<b>Diameter of stem</b>				
Vetch	2.81	2.81	2.68	2.80
Control	1.94	1.95	2.29	2.28
Red clover	2.58	2.48	2.54	2.56
LSD <sub>irrigation</sub> (p = 0.05)		0.23		0.24
LSD <sub>fertilization</sub> (p = 0.05)		0.24		0.13

D: Drip irrigation; S: Sprinkler irrigation

irrigation than in drip irrigation and this, in turn, caused a competition for water and nutrients between weeds and tobacco plants. In the drip system, water is only delivered to the root zone of each tobacco plant whereas in the overhead sprinkler system, the entire field receives a uniform amount of water resulting in more weed growth throughout the field. Similar results were reported by Boydak *et al.* (2007). Moreover, Antony and Singandhupe (2004) reported that drip irrigation is beneficial for capsicum plant (yield, better morphological character (plant height, number of branches, root fitness and root length)). Also, the highest plant height, number of branches and fruits per plant of aubergine were found in plots irrigated with drip system (Manjurantha *et al.*, 2004). In addition, Kassab *et al.* (2005) indicated that controlled surface irrigation surpassed the other two irrigation systems (sub-surface drip irrigation and surface drip irrigation) in sesame yield.

In general, the lowest stomatal conductance and photosynthetic rate were found in plots irrigated with sprinkler system. Antony and Singandhupe (2004) reported that at drip treatment, photosynthesis and conductance were maximum.

The highest correlation coefficient ( $r = 0.95^{***}$ , Table 6) between photosynthetic rate and stomatal conductance was found in Domokos region. Also, the stomatal conductance in Domokos was higher than those in Athens. The main reasons for higher stomatal conductance in Domokos may be attributed to temperature. As can be seen from (Fig. 1), the temperatures in Athens were always higher than those in Domokos. Finally, highest transpiration rates (Table 6) were found in plots irrigated with drip irrigation.



Table 9: Analysis of variance (F-ratio) of the two place data for morphological and physiological traits of tobacco plants

	Roots diameter	Width of leaves	Length of leaves	Diameter of stem
Source	-----F-ratio-----			
<b>Main effects</b>				
Green manure	6.89**	87.72***	85.46***	62.57***
Irrigation system	3.42 <sup>ns</sup>	2.14 <sup>ns</sup>	18.44***	0.02 <sup>ns</sup>
Place	27.50***	0.08 <sup>ns</sup>	29.71***	3.62 <sup>ns</sup>
<b>Interactions</b>				
Green manure × Irrigation system	1.21 <sup>ns</sup>	0.10 <sup>ns</sup>	1.71 <sup>ns</sup>	0.34 <sup>ns</sup>
Green manure × Place	6.15**	9.96***	8.85***	6.09**
Irrigation system × Place	4.13 <sup>ns</sup>	8.07***	7.94**	0.61 <sup>ns</sup>
	Stomatal conductance	Photosynthetic rate	Transpiration rate	
Source	-----F-ratio-----			
	----- (86 DAT) -----			
<b>Main effects</b>				
Green manure	73.53***	284.95***	100.63***	
Irrigation system	42.90***	60.65***	61.90***	
Place	126.38***	314.60***	216.57***	
<b>Interactions</b>				
Green manure × Irrigation system	18.27***	10.63***	21.68***	
Green manure × Place	31.99***	42.88***	73.73***	
Irrigation system × Place	28.49***	0.04 <sup>ns</sup>	6.21*	

\*, \*\*, \*\*\*: Significant at p = 0.05, 0.01 and 0.001, respectively; ns: not significant

### Analysis of Variance-Interactions

Irrigation methods, green manures were highly significant (Table 9,  $p < 0.001$ ) for physiological characteristics. Moreover, an irrigation x green manure interaction in physiological measurements was found. In contrast, there were no interaction between irrigation methods and green manures (Table 9) for morphological traits. Soil moisture availability is the main factor influencing nitrogen uptake by the root and transport to the leaf. Sifola and Postiglione (2003) reported that nitrogen uptake rate was increased by either irrigation or nitrogen fertilization. Moreover, Wang *et al.* (2003) and Ruggieior *et al.* (2004) reported that yield of tobacco increased significantly by the interaction between irrigation and the amount of nitrogen applied.

Green manures and drip irrigation has not been widely used in industrial crops. Presents results indicate that green manures and drip irrigation significantly affected tobacco growth characters.

### CONCLUSIONS

Present results indicate that irrigation system greatly affected the growth of tobacco. Drip irrigation increased the length of leaves. Also, there were no significant differences between the drip and sprinkler irrigation for root diameter. In general, the lowest stomatal conductance and photosynthetic rate were found in plots irrigated with sprinkler system. Moreover, physiological characteristics were significantly influenced by the green manures. The lowest stomatal conductance and photosynthetic rate were found in control plots. Also, the interaction between irrigation systems and green manures was not significant in all studied traits.

Final, the obtained results suggested that drip irrigation and green manure crops could be recommended for improving productivity of tobacco plants under similar conditions.

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