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ORIGINAL PAPER

EFFECT OF CHLORIDE CONTENT IN IRRIGATION WATER ON YIELD AND CHEMICAL COMPOSITION OF LEAVES OF THREE ORIENTAL TOBACCO (*NICOTIANA TABACUM* L.) CULTIVARS

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Abstract

Chloride is a micronutrient that has a major influence on the development of tobacco, more specifically its quality, that is on its chemical properties and on the overall structure of the leaves. A greenhouse pot experiment was carried out at the Agricultural University of Athens in 2018, in order to investigate changes in agronomic and quality properties of three Oriental tobacco cultivars in response to increased levels of chloride in irrigation water. The experimental layout was a two-factor factorial design of 3 x 4 [three Oriental tobacco cultivars: Basma Xanthi 81 (aromatic type), and Myrodato Agrinion 13B (neutral type), and Pieria Samson 53 (taste type and four chloride levels: 0, 30, 60 and 120 mg Cl^- dm^{-3}) with six one-pot replications. The results indicated that Oriental-type tobacco was affected by both chloride concentration in irrigation water and cultivars. The highest total number of leaves per plant (24.0) and yield of total cured product (29.15 g $plant^{-1}$) were found in Basma Xanthi 81 cultivar irrigated with 30 mg Cl^- dm^{-3}, while the highest middle leaf surface area (768.72 cm^2) was observed in Myrodato Agrinion 13B irrigated with 30 mg Cl^- dm^{-3}. In terms of qualitative characteristics of Oriental tobacco leaves, the highest chlorine content (28.16 g kg^{-1} DM) was found in Myrodato Agrinion 13B irrigated with the highest chloride level (120 mg Cl^- dm^{-3}). Concerning the nicotine content, the highest value (13.76 g kg^{-1} DM) was found in Basma Xanthi 81 cultivar irrigated with 30 mg Cl^- dm^{-3}. To conclude, the optimum chloride level in irrigation water is 30 Cl^- dm^{-3}. Among the cultivars, the aromatic cultivar Basma Xanthi 81 presented better yield and quality characteristics to increased levels of chloride in irrigation water. However, there is a clear need to continue this study in order to investigate the performance of several unexploited oriental tobacco cultivars.

Keywords: irrigation water salinity, leaf area, nicotine (NIC), oriental tobacco yield, reducing sugars (RS).

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INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is one of the most frequently used plants in mineral nutrition studies. Many essential chemical (e.g., nicotine, sugars, starch, total nitrogen, and mineral composition) and physical (e.g., combustibility, filling power, and equilibrium moisture content) characteristics are used to assess tobacco quality (Karaivazoglou et al. 2006). The physical and chemical characteristics of tobacco leaves are affected by genetics, agricultural practices, soil type and minerals, meteorological conditions, plant disease, stalk position, as well as harvesting and curing procedures. Changes in any of these factors can significantly alter the chemical composition of leaf and can therefore affect the quality of smoking (Tso 1972, Tso 1990).

Chlorine is generally recognized as an essential micronutrient, which is taken up very rapidly and in considerable amounts by tobacco plant (Rhoads 1975, Collins, Hawks 1993). It constitutes a valuable compound of tobacco leaves that affects quality factors such as color, moisture content, elasticity, and burning. Small amounts of chlorine, up to 2% in fertilizer, improve the yield, and certain quality factors such as color, moisture content, elasticity, burning, and keeping tobacco quality (McEvoy 1950). In contrast, high leaf chlorine content has a negative effect on burn rate as it decreases the fire holding capacity of the tobacco leaf. Moreover, aroma and taste are aggravated when chlorine increases, resulting in poor quality of tobacco (Karaivazoglou et al. 2006, Yamini et al. 2018).

Chlorine accumulation by tobacco plants in considerable amounts has been reported several times even without chloride application, but from chloride existed in soil, manure, or compost (Karaivazoglou et al. 2005). The amounts of chlorine in leaves vary depending on tobacco type, variety, and methods of fertilization, cultivation and harvesting, soil pH, etc. (Darvishzadeha et al. 2011). In addition, chloride is absorbed in large quantities at the beginning of the tobacco growing period (Ward 1941).

Oriental-type tobacco presents a special group of tobacco, which gives bouquet through the process of cigarettes blending. The main characteristics are the small leaves and the strong specific aroma. Many of the early brands of cigarettes were mostly or completely entirely made from oriental tobacco (Karaivazoglou et al. 2006).

Oriental tobacco is of national importance and is extremely popular with Greek farmers, as Greece is among the major Oriental tobacco producing countries in the world, and this tobacco type may use low fertility soils that are unproductive for other crops. Nowadays, high chloride concentration in soil and irrigation water is a growing concern in tobacco growing areas; however, there is limited research on the response to increasing soil chloride concentrations in terms of growth, yield, and quality. The present research study was conducted to investigate changes in agronomic and quality properties of three Oriental tobacco varieties in response to increased levels of chloride in irrigation water, in order to evaluate whether the three different types (aromatic, neutral, and taste) of Oriental tobacco cultivars respond differently to chloride concentrations, and to determine an acceptable chloride concentration in irrigation water.

MATERIALS AND METHODS

A greenhouse pot experiment was conducted from February to May 2018 at the Agricultural University of Athens (latitude 37°59′N, longitude 23°42′, 30 m from the sea surface), Greece, using three Oriental tobacco cultivars in order to determine the effect of four chloride levels (0, 30, 60 and 120 mg Cl^{-} dm^{-3}) in irrigation water. The cultivars were Basma Xanthi 81 of the aromatic type, Myrodato Agrinion 13B of the neutral type, and Pieria Samson 53 of the taste type. The experimental layout was a two-factor factorial of 3 x 4 (three Oriental tobacco cultivars and four chloride levels) with six one-pot replications. The daily minimum and maximum air temperature in the greenhouse during the growing period of Oriental tobacco are presented in Figure 1. The minimum and maximum relative humidity were 50% and 70%, respectively, and the plants were subjected to a natural day length ranging between 10-14h during the experiment (February-May).

The plastic pots were filled with 12 kg of a soil substrate consisting of one volume of clay loam soil, one volume of peat, and one volume of perlite. Some physicochemical characteristics of the substrate used are presented in Table 1. The soil type was a clay loam (28.7% clay, 33.9% silt,

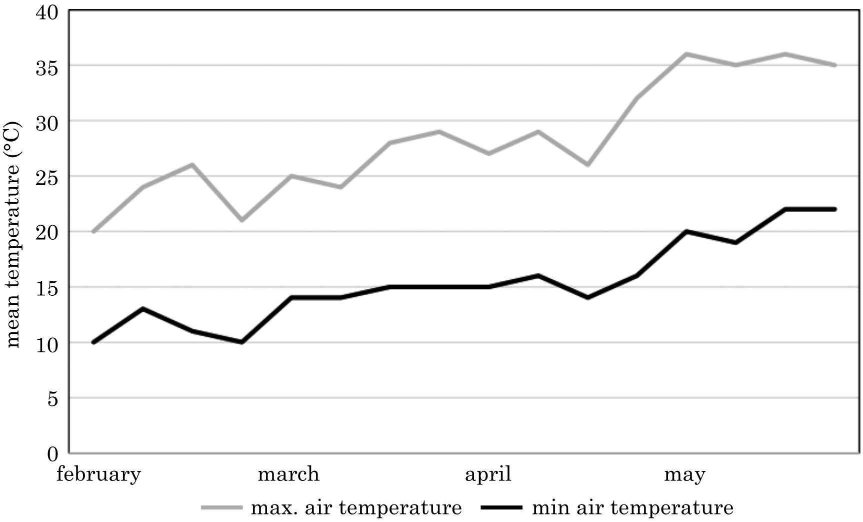

Fig. 1. Mean minimum and maximum daily air temperature in the greenhouse during the experiment (February-May, 2018)

Table 1

Physicochemical properties of soil substrate used in the experiment

Substrate properties	Soil, peat, and perlite (1:1:1)
Soil type	clay loam (28.7% clay, 33.9% silt, 37.4% sand)
pH (1:2 H_2O)	7.3
Organic matter	0.37(%)
$CaCO_3$	15.99(%)
P (Olsen)	9.95 (mg kg^{-1})
NO_3^-	104.3 (mg kg^{-1})
Na^+	110 (mg kg^{-1})

and 37.4% sand) (Bouyoucos 1962), with pH 7.3 (1:2 soil/water ratio); 0.37% organic matter content (determined by the wet-oxidation method of Walkley, Black 1934); 15.99% calcium carbonate ($CaCO_3$, determined by the Bernard calcimeter method of Muller, Gatsner 1971); Olsen P, 9.95 mg kg^{-1} (Olsen, Sommers 1982); nitrate (NO_3^-) content, 104.3 mg kg^{-1} (Keeney, Nelson 1982); and sodium (Na^+) concentration, 110 mg kg^{-1} (Roy 1956).

Ten seeds were sown in each plastic pot on 9th February 2018, and seedlings were thinned to one per pot after emergence, which was on 19th February 2018. The plants were irrigated with deionized water containing 0, 30, 60 and 120 mg Cl^- dm^{-3}. Chloride was added to the deionized water as $CaCl_2$. From 30 days after sowing (DAS) until the end of harvest (90 DAS), plants were irrigated once weekly by applying 2 dm^3 of salt solution to each pot. This quantity of water was not sufficient to cause leaching.

The plant growth-development was determined by measuring plant height and total number of leaves per plant at 40, 50, 60, 70, and 80 DAS. Moreover, the middle leaf surface area was calculated at 80 DAS with the following equation described by Moustakas, Ntzanis (1998):

$$S = k(L \cdot W),$$

where: S – leaf area, L – leaf length, W – leaf width, and k – 0.653, the empirical constant according to Moustakas, Ntzanis (1998).

Length (L) and width (W) of selected leaves were measured manually to the nearest 1 mm.

All plants were harvested by hand at 90 DAS. The harvested leaves were cured in the sun. The cured leaves were weighed to determine the yield of the cured product, and then the samples were dried at 80°C for 72 h, to constant dry weight, in a forced-air oven and ground in a Wiley mill using a 1 mm sieve. The samples were determined potentiometrically for chloride (method 963.05; AOAC 1990) using a chloride ion-selective electrode, ISE (Thermo Scientific Orion 9617BNWP; Orion Research Inc., Cambridge, Massachusetts, USA) in conjunction with a double-junction reference electrode (Thermo Scientific Orion 900200; Orion Research Inc., Cambridge, Massachusetts, USA) and a millivoltmeter (Orion 701A digital pH/mV meter; Orion Research Inc., Cambridge, Massachusetts, USA). Nicotine content (NIC) was determined by reaction with sulphanilic acid and cyanogen chloride using CORESTA recommended methods no. 35 (ISO/DIS 15152: 2003) (CORESTA 2010*a*). Nitrates were determined by reduction of nitrate to nitrite with hydrazinium sulphate in the presence of a copper catalyst, followed by reaction with sulphanilamide to form the diazo compound, method no. 36 (ISO/DIS 15157: 2003) (CORESTA 2015). Reducing sugars (RS) (as a sum of glucose, galactose and fructose) extracted with 5% acetic acid solution were determined by reaction with p-hydroxybenzoic acid hydrazide, method no. 38 (ISO/DIS 15154: 2003) (CORESTA 2010*b*).

Data were subjected to two-way analysis of variance (ANOVA) using the SigmaPlot 12 statistical software (Systat Software Inc., San Jose, CA, USA). The differences between means were separated using the Least Significant Difference (LSD) test. All comparisons were made at the 5% level of significance ($p<0.05$).

RESULTS AND DISCUSSION

The research results indicated that plant height of Oriental tobacco during the entire growing season (40, 50, 60, 70, and 80 DAS) was significantly affected by both different chloride concentrations in irrigation water and different cultivars, with the maximum value achieved at 80 DAS (Table 2). Concerning the effect of chloride, the mean values were the highest at chloride application amounts of 30 mg Cl^- dm^{-3} (100.7 cm) and 60 mg Cl^- dm^{-3} (99.2 cm) followed by chloride application amounts of 0 mg Cl^- dm^{-3} (93.5 cm) and 120 mg Cl^- dm^{-3} (89.4 cm). In an outdoor pot experiment with tobacco cultivars, Gul et al. (2006) found that all growth parameters increased with the initial dose of potassium chloride 4 mmol kg^{-1}, but then decreased with higher levels. Karaivazoglou et al. (2006) observed that the plant height increased in neutral cultivars of oriental tobacco as chloride in irrigation water increased from 10 mg dm^{-3} to 50 mg dm^{-3}. In regard to cultivars effect, the mean values were the highest in Basma Xanthi 81 (108.6 cm) and Myrodato Agrinion 13B (103.9 cm) plants followed by Pieria Samson 53 (74.6 cm). Darvishzadeh et al. (2011) reported that chloride uptake by Oriental tobacco varied with genotypes and suggested that chloride accumulation is genetically controlled.

The effects of the chloride concentration in irrigation water and of a cultivar on the total number of leaves of Oriental tobacco are presented

Table 2

Effect of chloride and cultivar on plant height (cm)

Chloride (mg $Cl^{-}\cdot dm^{-3}$)	Cultivar	Plant height (cm)				
		40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
0	Basma Xanthi 81	9.8	22.1	50.7	74.3	103.3
	Myrodato Agrinion 13B	9.1	17.5	42.1	56.1	93.5
	Pieria Samson 53	9.0	16.3	33.1	49.9	69.0
	mean:	9.3	18.6	42.0	60.1	88.6
30	Basma Xanthi 81	13.1	28.4	59.2	77.2	111.0
	Myrodato Agrinion 13B	11.6	25.2	47.6	64.0	110.0
	Pieria Samson 53	11.0	18.9	32.6	55.4	76.6
	mean:	11.9	24.2	46.5	65.5	99.2
60	Basma Xanthi 81	12.0	24.9	50.8	74.7	114.3
	Myrodato Agrinion 13B	12.3	21.9	45.4	62.7	110.3
	Pieria Samson 53	9.8	20.2	35.2	52.8	77.4
	mean:	11.4	22.3	43.8	63.4	100.7
120	Basma Xanthi 81	13.7	24.8	52.3	67.4	105.9
	Myrodato Agrinion 13B	11.6	16.9	37.1	61.8	101.6
	Pieria Samson 53	10.3	16.9	29.0	46.7	75.5
	mean:	11.9	19.5	39.5	58.6	94.3
	Basma Xanthi 81	12.2	25.1	53.3	73.4	108.6
	Myrodato Agrinion 13B	11.2	20.4	43.1	61.2	103.9
	Pieria Samson 53	10.0	18.1	32.5	51.2	74.6
	mean:	11.1	21.2	43.0	61.9	95.7
$F_{chloride}$		4.06** (LSD = 1.64)	7.68*** (LSD = 2.05)	5.05** (LSD = 3.20)	3.41* (LSD = 3.49)	5.45** (LSD = 5.943)
$F_{cultivar}$		5.25** (LSD = 1.43)	19.86*** (LSD = 1.46)	83.58*** (LSD = 3.46)	56.82*** (LSD = 4.28)	91.43*** (LSD = 5.75)
$F_{chloride\ x\ cultivar}$		1.11^{ns}	1.12^{ns}	1.70^{ns}	0.83^{ns}	0.75^{ns}

F-test ratios are from ANOVA. Significant at *, ** and *** indicate significance at p=0.05, 0.01 and 0.001, respectively, and ns – not significant. The LSD test (p=0.05) values for chloride and cultivar are also presented.

in Table 3. The analysis of variance generally revealed that the total leaf number was significantly affected by the chloride concentration, and the maximum values were achieved at 80 DAS. Specifically, the greatest value (22.5) was found in plants irrigated with 30 mg Cl^{-} dm^{-3}, followed by 60 mg Cl^{-} dm^{-3} (19.8) and 0 mg Cl^{-} dm^{-3} (19.3). The lowest value (17.8) was observed in plants irrigated with the highest chloride concentration (120 mg dm^{-3}). An increase in the chloride concentration above 60 mg dm^{-3} exerted significant stress effects on tobacco plants. This result agrees with the claim that tobacco is a chloride-intolerant plant (Wen et al. 2004).

Table 3

Effect of chloride and cultivar on the total number of leaves per plant

Chloride (mg Cl·dm⁻³)	Cultivar	Total number of leaves				
		40 DAS	50 DAS	60 DAS	70 DAS	80 DAS
0	Basma Xanthi 81	6.0	8.2	9.8	16.6	22.2
	Myrodato Agrinion 13B	5.6	7.1	8.0	13.8	18.4
	Pieria Samson 53	5.4	7.3	8.2	13.2	17.2
	mean:	5.7	7.5	8.7	14.5	19.3
30	Basma Xanthi 81	6.7	8.7	11.6	16.6	24.0
	Myrodato Agrinion 13B	6.6	9.2	11.0	14.6	23.2
	Pieria Samson 53	5.7	7.5	10.1	14.2	20.4
	mean:	6.3	8.5	10.9	15.1	22.5
60	Basma Xanthi 81	6.3	7.6	9.8	16.1	22.3
	Myrodato Agrinion 13B	5.7	7.7	8.7	13.8	19.3
	Pieria Samson 53	5.4	7.3	7.8	14.0	17.7
	mean:	5.8	7.5	8.8	14.6	19.8
120	Basma Xanthi 81	6.2	8.1	9.3	16.2	19.7
	Myrodato Agrinion 13B	5.7	7.0	8.8	14.1	17.8
	Pieria Samson 53	4.7	6.7	7.5	13.2	15.8
	mean:	5.5	7.3	8.5	14.5	17.8
	Basma Xanthi 81	6.3	8.2	10.1	16.4	22.1
	Myrodato Agrinion 13B	5.9	7.8	9.1	14.1	19.7
	Pieria Samson 53	5.3	7.2	8.4	13.7	17.8
	mean:	5.8	7.7	9.2	14.7	19.8
$F_{chloride}$		3.98* (LSD = 0.55)	3.69* (LSD = 0.80)	16.24*** (LSD = 0.86)	1.44^{ns}	18.08*** (LSD = 1.65)
$F_{cultivar}$		11.17*** (LSD = 0.45)	3.95* (LSD = 0.69)	13.62*** (LSD = 0.50)	48.27*** (LSD = 0.58)	27.72*** (LSD = 1.41)
$F_{chloride\ x\ cultivar}$		0.53^{ns}	1.26^{ns}	0.67^{ns}	0.57^{ns}	0.61^{ns}

F-test ratios are from ANOVA. Significant at *, ** and *** indicate significance at p=0.05, 0.01 and 0.001, respectively, and ns – not significant. The LSD test (p=0.05) values for chloride and cultivar are also presented.

The total number of leaves was significantly affected by different Oriental tobacco cultivars. The highest number (22.0) was found in Basma Xanthi 81, followed by Myrodato Agrinion 13B (19.7) and Pieria Samson 53 (17.8). Karaivazoglou et al. (2006) demonstrated that the total number of leaves in Oriental tobacco plants was a cultivar-specific characteristic, and was not affected by a chloride concentration up to 50 mg Cl^- dm^{-3} in irrigation water.

The middle leaf surface area was influenced by both the chlorine concentration and the cultivars (Table 4). The highest values of leaf area (768.72, 642.98, and 525.61 cm^2 in Myrodato Agrinion 13B, Pieria Samson 53,

Table 4

Effect of chloride and cultivar on the middle leaf surface area (cm^2) and yield of total cured product (g $plant^{-1}$)

Chloride (mg $Cl^- dm^{-3}$)	Cultivar	Middle leaf surface area (cm^2)	Yield of total cured product (g $plant^{-1}$)
0	Basma Xanthi 81	365.70	18.40
	Myrodato Agrinion 13B	555.99	20.63
	Pieria Samson 53	560.62	12.79
	mean:	494.10	17.27
30	Basma Xanthi 81	525.61	29.15
	Myrodato Agrinion 13B	768.72	22.61
	Pieria Samson 53	642.98	19.62
	mean:	645.77	23.79
60	Basma Xanthi 81	499.47	21.34
	Myrodato Agrinion 13B	733.34	16.42
	Pieria Samson 53	632.52	17.64
	mean:	621.78	18.47
120	Basma Xanthi 81	345.83	16.12
	Myrodato Agrinion 13B	542.57	13.51
	Pieria Samson 53	485.89	16.66
	mean:	458.10	15.43
	Basma Xanthi 81	434.15	21.25
	Myrodato Agrinion 13B	650.16	18.29
	Pieria Samson 53	580.50	16.68
	mean:	554.94	18.74
$F_{chloride}$		2.53* (LSD = 168.21)	4.49* (LSD = 4.052)
$F_{cultivar}$		5.20* (LSD = 138.68)	3.50* (LSD = 2.245)
$F_{chloride\ x\ cultivar}$		0.11^{ns}	0.53^{ns}

F-test ratios are from ANOVA. Significant at *, ** and *** indicate significance at p=0.05, 0.01 and 0.001, respectively, and ns – not significant. The LSD test (p=0.05) values for chloride and cultivar are also presented.

and Basma Xanthi 81, respectively) were observed in plants irrigated with 30 mg Cl^- dm^{-3}. Regarding the influence of a cultivar, the highest leaf area values were found in the neutral cultivar Myrodato Agrinion 13B (650.16 cm^2) and the taste cultivar Pieria Samson 53 (580.50 cm^2), while the lowest value was found in the aromatic cultivar Basma Xanthi 81 (434.15 cm^2). In general, aromatic cultivars are thin and delicate, with small leaves; they ensure very fine, smooth, and rich aromatic smoking, and a characteristic deep taste, while neutral cultivars are thick-bodied cultivars, with bigger leaves and little oil or aroma, and finally taste cultivars have a pleasant sweet taste as well as characteristic heart-shaped leaves, and a naked stem (Sficas 1970, Lolas et al. 1983).

The yield of total cured product was also significantly affected by different chlorine concentrations in irrigation water and Oriental cultivars. The highest yield (23.79 g plant^{-1}) was found in plants irrigated with 30 mg Cl^- dm^{-3}, followed by 60 mg Cl^- dm^{-3} (18.46 g plant^{-1}), 0 mg Cl^- dm^{-3} (17.27 g plant^{-1}), and 120 mg Cl^- dm^{-3} (15.43 g plant^{-1}). In regard to the cultivars, the highest yield (21.25 g plant^{-1}) was recorded in Basma Xanthi 81, while the lowest was obtained from Pieria Samson 53 (16.68 g plant^{-1}). The cured leaf yield increase induced by a chloride concentration was associated with the stimulation of higher cell size, leaf growth and leaf number (Franco-Navarro et al. 2015). Positive yield response of Oriental tobacco to chloride concentration up to 50 mg Cl^- dm^{-3} in irrigation water was reported by Karaivazoglou et al. (2006). Similarly, a small amount of chloride added to soil had a positive effect on cured leaf yield of Virginia tobacco (Jones 1984). On the other hand, several studies in Burley (Fuqua et al. 1976) and Virginia tobacco (Collins, Hawks 1993) showed no significant differences in cured leaf yield when different quantities of chloride were applied to the soil. In addition, Peele et al. (1960) demonstrated that the yield response of flue-cured tobacco to different chloride levels (from 5 to 225 mg dm^{-3}) in irrigation water was unaffected.

Chloride toxicity in green tobacco has been described as causing thickened yellow leaves, exceedingly brittle with leaf margins curling upward (McCants, Woltz 1967). According to Karaivazoglou et al. (2005), the early premature yellowing of Virginia tobacco leaves could be used as an indication of high chloride concentration when the nitrogen concentration in soil is sufficient to satisfy plant nitrogen nutrition needs. In the present study, premature fading green to yellow leaves in Oriental tobacco plants irrigated with the highest chloride level (120 dm^{-3}) could be attributed mainly to chloride toxicity, and not to reduced nitrogen concentration.

As expected, increasing chloride in irrigation water increased the chloride content of Oriental tobacco leaves. The highest values of leaf chlorine content (28.16, 24.53, and 24.48 in g kg^{-1} DM in Myrodato Agrinion 13B, Pieria Samson 53, and Basma Xanthi 81) were found in plants irrigated with 120 mg Cl^- dm^{-3} (Table 5). These results are in full accordance with other studies that demonstrated a significant positive correlation between chloride levels in irrigation water or in soils and leaf chlorine content in Burley (Johnson, Sims 1986), Virginia (Metochis, Orphanos 1990, Karaivatzoglou et al. 2005) and Oriental tobacco (Karaivatzoglou et al. 2006). The highest concentration of chlorine is found in the lower leaves, and it decreases progressively to the top of the plant (data not shown). This could be explained by the fact that chlorine accumulates in plant tissues as they age, and as a result the content of chlorine in older lower leaves was higher than in younger upper leaves (Metochis, Orphanos 1990, Flower 1999). Contrariwise, Karaivatzoglou et al. (2006) reported that in Oriental tobacco harvested in five primings at weekly intervals, the leaf chlorine content was highest in the upper leaves and decreased gradually from the upper (fifth priming)

Table 5

Effect of chloride and cultivar on chlorine, nicotine, reducing sugars, and nitrates

Chloride (mg Cl$^-$dm^{-3})	Cultivar	Chlorine (g kg^{-1} DM)	Nicotine (g kg^{-1} DM)	Reducing sugars (g kg^{-1} DM)	Nitrates (g kg^{-1} DM)
0	Basma Xanthi 81	13.67	11.10	60.70	1.33
	Myrodato Agrinion 13B	14.35	7.74	52.16	2.35
	Pieria Samson 53	13.08	5.33	49.53	1.58
	mean:	13.70	8.06	54.13	1.75
30	Basma Xanthi 81	14.35	13.76	81.01	0.94
	Myrodato Agrinion 13B	14.97	7.79	63.47	1.58
	Pieria Samson 53	14.69	5.42	72.77	1.53
	mean:	14.67	8.99	72.42	1.35
60	Basma Xanthi 81	18.96	10.95	93.65	0.93
	Myrodato Agrinion 13B	22.56	6.85	88.42	0.89
	Pieria Samson 53	19.89	4.78	96.21	1.23
	mean:	20.47	7.53	92.76	1.02
120	Basma Xanthi 81	24.48	10.02	112.01	0.81
	Myrodato Agrinion 13B	28.16	6.81	99.06	0.83
	Pieria Samson 53	24.53	4.14	102.03	0.99
	mean:	25.72	6.99	104.37	0.88
	Basma Xanthi 81	17.87	11.46	86.84	1.00
	Myrodato Agrinion 13B	20.01	7.30	75.78	1.41
	Pieria Samson 53	18.05	4.92	80.14	1.33
	mean:	18.64	7.89	80.92	1.25
F_{chloride}		20.09*** (LSD = 0.354)	14.52*** (LSD = 15.45)	6.69** (LSD = 0.61)	37.33*** (LSD = 1.65)
F_{cultivar}		41.93*** (LSD = 0.231)	25.67*** (LSD = 17.76)	55.11*** (LSD = 0.87)	2.53^{ns}
$F_{\text{chloride x cultivar}}$		0.96^{ns}	1.09^{ns}	1.01^{ns}	0.43^{ns}

F-test ratios are from ANOVA. Significant at *, ** and *** indicate significance at p=0.05, 0.01 and 0.001, respectively, and ns – not significant. The LSD test (p=0.05) values for chloride and cultivar are also presented.

to the lower leaves (first priming) of the plant. The same authors explained that this discrepancy is probably related to the harvesting method used, with chloride added continuously to the soil with irrigation water, resulting in higher chlorine accumulation of upper leaves than the lower leaves.

In our study, the accumulation rate of chlorine in leaves also depended on cultivars. The chlorine content recorded in Myrodato Agrinion 13B (20.01 g kg^{-1} DM) was greater than in Pieria Samson 53 (18.05 g kg^{-1} DM) and Basma Xanthi 81(17.87 g kg^{-1} DM). Indeed, Karaivatzoglou et al. (2006) observed that in Oriental tobacco plants irrigated with the same chloride level, a neutral type had higher chlorine accumulation in leaves than an aromatic one. In addition, Bozhinova (2012) observed differences in the leaf chlorine concentration and accumulation rate of chloride between six Virginia tobacco cultivars.

For Oriental type of tobacco, it is considered generally acceptable to contain up to 15 g kg^{-1} DM of chloride in leaves, as a high leaf chloride content has a negative effect on the fire-holding capacity of the tobacco leaf (Karaivatzoglou et al. 2006, Darvishzadeh et al. 2011). Based on the results collated in Table 5, it is preferable to use irrigation water with a chloride concent up to 30 mg dm^{-3} because at this level the chloride concentration in Oriental tobacco leaves remained up to 15 g kg^{-1} DM of chloride.

The effect of chloride on nicotine (NIC) content of leaves was significant and showed inconsistent trend (Table 5). The highest content (8.98 g kg^{-1} DM) was found in plants irrigated with 30 mg Cl^- dm^{-3}, followed by 0 mg Cl^- dm^{-3} (8.05 g kg^{-1} DM), 60 mg Cl^- dm^{-3} (7.53 g kg^{-1} DM), and 120 mg Cl^- dm^{-3} (6.99 g kg^{-1} DM). Similar inconsistent results and a significant effect of chloride on NIC content in Virginia and Oriental tobacco have been reported by Collins, Hawks (1993) and Karaivatzoglou et al. (2006), respectively. In regard to the effect of cultivars, the highest NIC content (11.46 g kg^{-1} DM) was found in Basma Xanthi 81, while the lowest (4.92 g kg^{-1} DM) in Pieria Samson 53. Significant differences on NIC content among Oriental-type cultivars have also been reported by Bilalis et al. (2015). In addition, Karaivatzoglou et al. et al. (2006) also observed that in Oriental tobacco plants irrigated with the same chloride level, the aromatic type (Basma) had higher NIC content in leaves than the oriental type.

As with NIC content, reducing sugars (RS) concentration also constitutes a significant indicator of smoking quality. The effects of the chloride concentration in irrigation water and cultivar on the RS concentration of Oriental--type tobacco leaves are shown in Table 5. Analysis of variance revealed that the RS concentration was significantly affected by chloride concentration. More specifically, the highest value (104.37 g kg^{-1} DM) was observed in plants irrigated with 120 mg Cl^- dm^{-3}, followed by 60 mg Cl^- dm^{-3} (92.76 g kg^{-1} DM) and 30 mg Cl^- dm^{-3} (72.41 g kg^{-1} DM). The lowest value (54.13 g kg^{-1} DM) was recorded in plants irrigated with the lowest chloride concentration (0 mg dm^{-3}). Increasing chloride level enhanced soluble carbohydrate concentration, and is mainly due to the fact that carbohydrate plays a crucial role in maintaining osmotic balance in plants, which are exposed to salt stress, and therefore protects plants from adverse salt effects (Datta, Kulkarin 2014). Positive correlations between chlorine content and sugar content were also observed by others (Peele et al. 1960, Langeroodi et al. 2017). In the present research, cultivar substantially influenced the levels of reducing sugars, and not only NIC content in tobacco leaf, confirming the important role of genotype on the quality characteristics of tobacco (Bilalis et al. 2015). The highest value (86.84 g kg^{-1} DM) was observed in Basma Xanthi 81, whereas the lowest (75.78 g kg^{-1} DM). was found in Myrodato Agrinion 13B cultivar.

Regarding the nitrate content, it was only affected by the chloride content in irrigation water and decreased with the increasing chloride levels. The highest values (2.53, 1.58, and 1.33 g kg^{-1} DM in Myrodato Agrinion 13B, Pieria Samson 53, and Basma Xanthi 81, respectively) were found in plants irrigated with 0 mg Cl^{-} dm^{-3}, while the lowest values (0.99, 0.83, and 0.81 g kg^{-1} DM in Pieria Samson 53, Myrodato Agrinion 13B, and Basma Xanthi 81, respectively) were recorded in plants irrigated with the highest chloride level (120 mg dm^{-3}). Earlier results indicated that nitrates (NO_3^-) or chloride (Cl^-) inhibited the uptake of the other anion during the growth of the tobacco (Fuqua et al. 1974, 1976). In the present study, a significant negative correlation was observed between nitrate and chloride content (r=-0.4201, p=0.0213) confirming the above conclusion.

CONCLUSION

The results indicated that the yield of Oriental-type of tobacco was affected by both the chloride concentration in irrigation water and the cultivars. The highest total number of leaves per plant and yield of total cured product were found in Basma Xanthi 81 cultivar irrigated with 30 mg Cl^{-} dm^{-3}, while the highest middle leaf surface area was observed in Myrodato Agrinion 13B irrigated with 30 mg Cl^{-} dm^{-3}. In terms of qualitative characteristics of Oriental tobacco leaves, NIC content had the tendency to decline in plants irrigated with water containing more than 30 mg Cl^{-} dm^{-3}. Moreover, an increase of the chloride level in irrigation water simultaneously increased the chlorine content and RS concentration, but reduced the nitrate content in leaves. To conclude, the optimum chloride level in irrigation water is 30 mg dm^{-3}. The distribution of chloride concentrations in leaves under high chloride levels in irrigation water found in this research study provides useful information for tobacco manufacturers on the formation of various cigarette blends. The cultivars evaluated herein demonstrated different chloride accumulation rates. Among the cultivars, the aromatic cultivar Basma Xanthi 81 produced better yield and quality characteristics in response to increased levels of chloride in irrigation water in comparison to the other two studied cultivars (Pieria Samson 53, Myrodato Agrinion 13B). However, there is a clear need to continue this study in order to investigate the performance of several unexploited oriental tobacco cultivars.

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