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Nutrition Management and Planting Arrangement Improve Growth Indices and Yield of Two Virginia Tobacco (*Nicotiana tabacum* L.) Cultivars

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ABSTRACT

Tobacco (*Nicotiana tabacum* L.) is a significant cash crop in Iran. It serves as a source of income for smallholder farmers and commercial fields. In order to investigate the growth indices and yield of two Virginia tobacco cultivars an experiment was conducted in a split factorial based on RCBD design with eight treatments and three replicates during the 2020 cropping seasons. The treatments consisted of two application methods of N and K fertilizer (soil and foliar application), planting arrangement (double- and single-row) in the main plot and two Virginia tobacco cultivars (TC100 and NC100) in the sub-plot. Results showed that the highest value of tobacco leaf area index (LAI), crop growth rate (CGR), relative growth rate (RGR), and total dry matter (TDM) were recorded where TC100 cultivar planted in double-row spacing and sprayed with N and K nutrients. Conversely, the lowest values of LAI, CGR, RGR and TDM were recorded where the NTC100 cultivar was planted in single-row spacing and received soil application of N and K. The overall results indicated that double-row and foliar application of N and K fertilizers could improve tobacco growth indices and ultimately its yield.

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1. Introduction

Tobacco (*Nicotiana tabacum* L.) is an herbaceous annual or perennial plant in the Solanaceae family that is grown for its leaves. Tobacco is cultivated in about 3.9 million hectares of which 60% is flue-cured, 13% is burley, and 12% is oriental (Mantesa *et al*., 2019). *N. tabacum* L. and *N. rustica* L. are the important commercial species of this family which is called the gold leaf plants in India (Patel *et al*., 2020). Albeit, leaves are the commercial portion of the plant, tobacco seeds comprise 38% of nonedible oil and therefore it could be a suitable candidate for diesel fuel (Darvishzadeh and Khalifani, 2022). Tobacco yield is affected by both climatic conditions and growing methods (Tabaxi *et al*., 2021).

Plant growth analysis is an explanatory, holistic, and integrative approach to interpreting plant form and function. It uses simple primary data in the form of weights, areas, volumes, and contents of plant components to investigate the processes within and involving the whole plant (Sugar *et al*., 2017). Several traits could be beneficially used for growth analyses. Some indices like net assimilation rate (NAR), crop growth rate (CGR), and relative growth rate (RGR) are physiological components because they are a measure of whole-plant daily net photosynthetic rate weighted by the rate of change in plant carbon content. The others as specific leaf area (SLA) and specific leaf weight (SLW) are considered morphological components because they are determined by leaf dry matter concentration and leaf thickness or measure the allocation of biomass to leaves vs. other plant parts (Çakir and Çebi, 2010). The dry weight of the plant is a function of the amount of radiation absorbed during

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the growth period. The amount of radiation absorbed by the plant completely depends on the LAI and the growth of the plant canopy (Mahakosee *et al*., 2022). In research, Bilalis et al. (2015) showed that less distance between plants leads to a decrease in size, mass weight, leaf thickness, and leaf weight (LW) per leaf area (LA) unit. However, high densities have increased the yield in some tobacco cultivars. Hence, Greek farmers tend to increase the spacing between planting rows to produce larger leaves (Bilalis *et al*., 2015). Rabnawaz Khan et al. (2018) showed that less row spacing leads to less LA and yield due particularly to increased competition between plants. Also, this research showed that increasing intra-row spacing from 45 cm to 50 cm and inter-row spacing from 90 cm to 120 cm increased the LA because enough space, light, and nutrients were provided to the plant. By comparison, increasing the distance between the plants in the row increases LA in tobacco by reducing the competition between plants for environmental resources. The CGR represents the amount of dry matter accumulation of plants in a certain period, time per unit of the land surface, and its value is the highest when the LAI is at the desired level, and then it decreases with shading and aging of the leaves (Kazemeini *et al*., 2010). Therefore, the growth rate of the crop is dependent on the LAI and it shows the efficiency of the canopy and absorption of sunlight on the other hand, it is effective in the process of increasing dry matter accumulation (Eyni-Nargeseh *et al*., 2020). Wang et al. (2003) reported that providing more N fertilizer to the plant increases the speed of LA expansion in the early growing season and causes early closure of the crop canopy. Therefore, increases in RGR, LAI, and leaf area duration (LAD) in the plant as a result of increasing the light penetration into the plant canopy resulted in increasing yield (Wang *et al*., 2003). Increasing the LAI is one of the ways to increase the photosynthesizing surface and guarantee tobacco performance in low light intensities. Therefore, compensation of low photosynthesis per unit of LA is considered a characteristic of leaves placed in the shade (Umesh *et al*., 2023). Adaptive plant responses to low radiation include an increase in LA ratio, leaf-to-stem weight ratio, stem length, a decrease in leaf thickness, and root growth relative to shoot growth (Wu *et al*., 2017).

More of the studies carried out recently across the world are related to the assessment of the effect of salinity, fertilizers, or other agricultural inputs on physiologically based parameters of wheat, maize, and soya crops (Çakir and Çebi, 2010). Only a few of them discuss the effects of N and K nutrition management and modern and conventional planting arrangement on physiological components such as CGR, RGR, and LAI of the mentioned crops, but almost no data exist for flue-cured Virginia tobacco. Therefore, the purpose of this experiment was to investigate the effect of nutrition management and planting arrangement on some growth indices of two Virginia tobacco cultivars in order to increase the quantitative and qualitative yield and increase the income of tobacco farmers.

2. Materials and methods

A field experiment was conducted in the Tirtash Research and Training Center located at 36° 43´ northern latitude and 53° 45´eastern longitude, in Mazandaran province, Iran in 2020. The experiment was laid out in a factorial split plot based on a randomized complete block design with three replications and eight treatments. The main plot includes two top dressing of N and K fertilizer (soil or foliar application), planting arrangement (Fig. 1) in two methods (single-row and double-row spacing) and subplots were two varieties of Virginia tobacco (TC100 and NC100). Some soil physical and chemical characteristics are presented in Table 1. The tobacco seeds were sown at the end of March 2020 and healthy seedlings were transplanted to the experimental plots in the last week of May 2020 when they attained a height of 15-20 cm. Irrigation operations were done according to different levels of soil moisture. Weeding was done manually after the establishment of the crop. The topping was done when more than 50% of the heading was completed in each subplot.

The fertilizer doses were considered according to the soil analysis (Table 1). All triple superphosphate was used after planting as a source of P fertilizer. One second of the urea (as a N) and potassium sulfate (as a K) fertilizers were added to the soil before transplanting. The remaining portion of both N and K fertilizers was divided into three stages and applied every 15 days after transplanting (DAT) either as a soil application or foliar spraying. Foliar spraying was done at the rate of 3 kg per 1000 L water. In order to determine the green yield of tobacco, in the early hours of the morning, ripe leaves (Volado, Seco and Ligero leaves) were harvested in each field, and then weighed with a digital scale with an accuracy of one thousandth, and then transferred to the warm house. Then green yield for each harvest was calculated separately and added at the end.

Table 1. Some physical and chemical properties of the studied soil.

Property	Unit	Value
Soil depth	cm	0-30
Textural class	-	Silt loam
Electrical Conductivity (EC)	ds/m	0.50
Wilting Point (WP)	%	21.0
Field Capacity (FC)	%	25.5
pH	-	7.30
Organic Carbon (OC)	%	0.57
Total Neutralizing Value (T.N.V.)	%	21.0
Total N	%	11.5
Available P	mg/kg	20.0
Available K	mg/kg	220

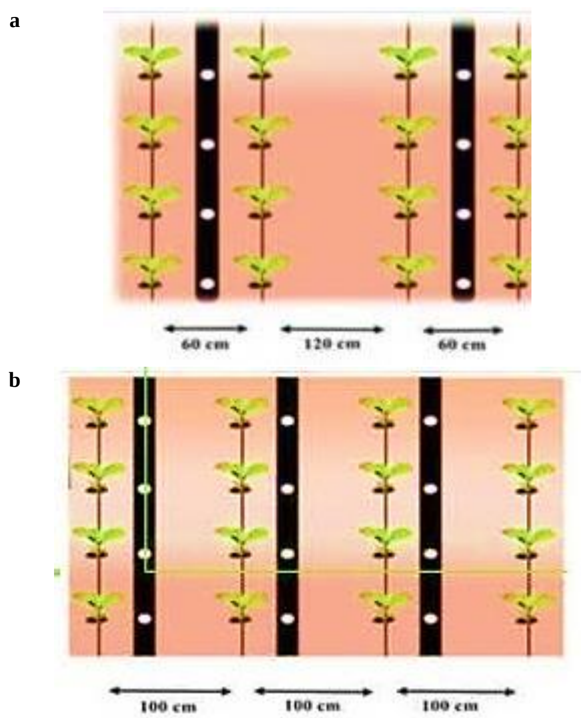

Figure 1. Double-row (a) and Single-row (b) cultivation

To evaluate the physiological growth indices, two plants were gently harvested from each plot. These samplings were started 30 DAT and leaves were harvested six times every 15 days intervals. After transferring the samples to the laboratory, the length and width of two leaves of the lower leaves of the plant (the lower leaves of the largest and widest leaves of each plant) were measured with a meter, and then the leaf area was calculated using Eq. 1 (Salvati, 2000).

$$Leaf\ area = Length\ leaf \times Width\ leaf \times 0.57 \quad (1)$$

After that, the leaves were separated from the stems and then weighed by placing them in an oven at a temperature of 60 degrees for 48 hours.

The three-parameter Gaussian equation (Eq. 2) was fitted to the LAI data, and the plant LA was estimated during the growth period (Steinmaus and Norris, 2002).

$$LAI(t) = a \exp\left[-0.5\left(\frac{x - x_0}{b}\right)^2\right] \quad (2)$$

t, time in days; LAI (t), estimated LAI; a, maximum LAI during the period; b is the time when the leaf surface index increases exponentially after this period; x0, is the time when the plant has the maximum LAI. To calculate the total dry matter parameter (TDM), the three-parameter sigmoid (Eq. 3) was fitted to the cumulative dry weight data (Steinmaus and Norris, 2002).

$$TDM = \frac{a}{1 + \exp\left[-b(t-m)\right]} \quad (3)$$

t, time in days; TDM, cumulative dry weight of the plant at time t; a, the maximum cumulative dry weight of the plant; b, dry matter increase slope; and m is the time when the plant has the highest growth rate or dry matter increase.

$$RGR = b\left(1 - \frac{TDM}{a}\right) \quad (4)$$

$$CGR = b \times TDM\left(1 - \frac{TDM}{a}\right) \quad (5)$$

The data collected during field and laboratory investigations were analyzed statistically using the analysis of variance (ANOVA) technique and means were compared using the least significant difference (LSD) test by the SAS (SAS Institute, 2004) software version 9.1. Multiple regression analysis was performed using Sigma Plot 11 software.

3. Results and discussion

3.1. Leaf area index (LAI)

Results showed that the changing trend of tobacco LAI was described with the three-parameter Gaussian equation in all treatments. This equation with high accuracy (R^2 = 0.86-0.99) was able to describe the changes in tobacco LAI during the growing seasons (Data not shown). LAI increased until the stage of physiological maturity and then decreased with increasing shading and decreasing light penetration into the canopy, decreasing the photosynthetic activity of leaves (Fig. 2).

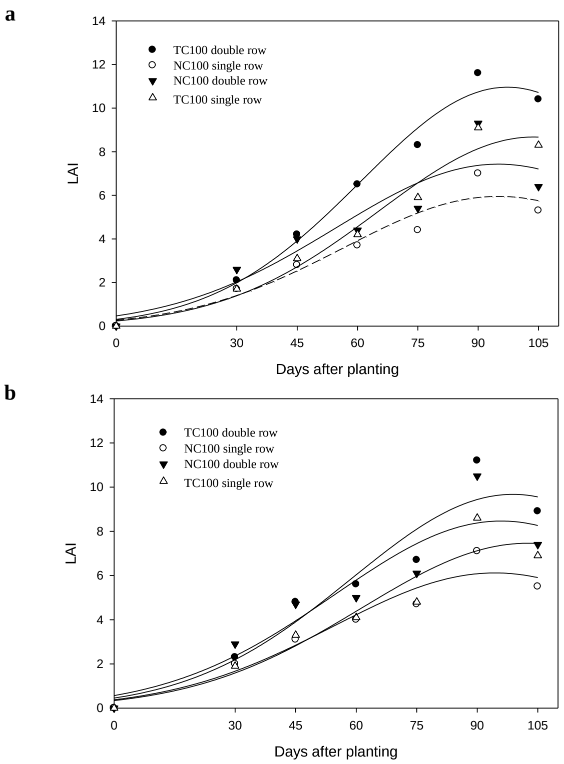

Figure 2. The trend of changes in LAI of NC100 and TC100 tobacco cultivars in single-row and double-row spacing in the method of foliar application (a) and soil application (b) of topdressing fertilizer.

The highest LAI value (10.96) was obtained when the TC100 cultivar was planted in double-row spacing and sprayed foliar. By comparison, the lowest value (LAI=5.95) was recorded in the NTC100 cultivar with conventional single-row spacing and soil application of N and K (Fig. 2). Similarly, Tang et al. (2020) and Mantesa et al. (2019) reported that increasing plant density increased yield and LAI in flue-cured tobacco, but decreased leaf quality. Also, some researches revealed that less distance between plants leads to reduced LAI (Bilalis *et al*., 2015; Kalaji *et al*., 2012). Zhang et al. (2021) reported that increasing LAI due to shading is one of the ways to increase the photosynthesized surface in order to guarantee proper yield in low light intensities. The maximum value of tobacco LAI in the TC100 cultivar and the foliar application was higher than in NC100 in both methods of fertilizer application (Fig. 2). In the conventional single-row spacing and soil application fertilizer, the maximum LAI of the NC100 cultivars were 14% lower than TC100 (Data not shown). Previous studies have shown that a simple way to increase LAI is to increase the density per unit area (Sugar *et al*., 2017; Tang *et al*., 2020). Also, in the foliar application at both single- and double-row spacing, the maximum LAI of the TC100 cultivar was 13 and 58% higher than NC100 (Data not shown). In the methods of soil and foliar application of top-dressing fertilizer in the conventional single-row spacing, the maximum LAI was recorded in NC100 by 8.75 and 6.35, respectively.

3.2. Total dry matter (TDM)

The changes in TDM in two cultivars of tobacco to NK nutrition and planting arrangement were described by the three-parameter sigmoid equation (Fig. 3 and 4; R^2 = 0.96-0.99). The highest TDM (3388 kg/ha) was obtained when the TC100 cultivar was planted in modern double-row spacing and foliar sprayed with NK fertilizer (Fig. 3a) while the minimum TDM (1337 kg/ha) was recorded in the NC100 cultivar when planted in conventional single-row spacing and soil application of NK fertilizer (Fig. 3b). Despite many reports of a decrease in dry weight due to increased plant density (e.g. Alvarenga *et al*., 2005; Yang *et al*., 2007) these results showed that with increasing plants density in double-row spacing the tobacco TDM was increased. This increase might be due to longer LAD at the shading treatments (Lambers *et al*., 1998). Also, the time to reach the peak value of TDM occurred earlier (48.6 days) in the superior treatment while this period was longer (81.2 days) in the inferior treatment (Data not shown). A similar pattern of TDM changes can be seen in the tobacco growing season in all examined treatments. At the beginning of growth, due to the smaller size of the plants, there was no significant difference between TDM trends in various treatments (Fig. 3 and 4). During most of the growing seasons, TDM in the two types of fertilizer application in both planting arrangements was higher for TC100 than NC100 cultivar (Fig. 3 and 4).

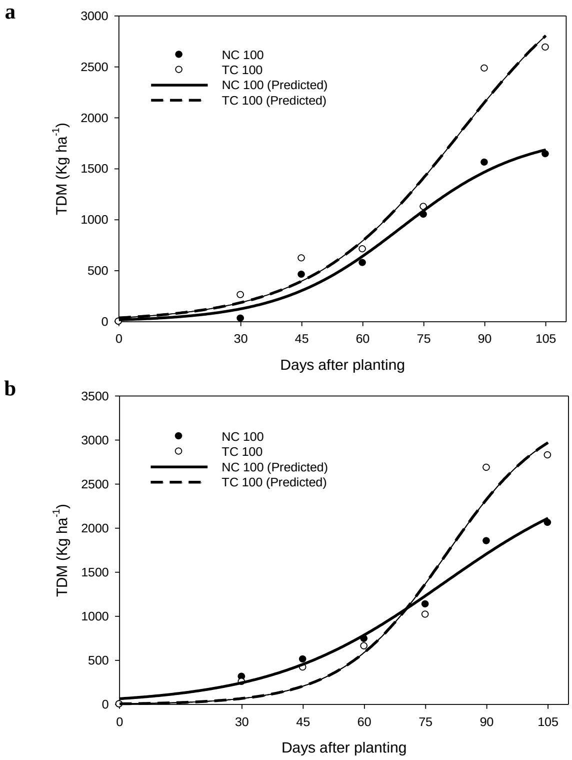

Figure 3. The trend of changes in TDM of NC100 and TC100 tobacco cultivars and the foliar application method of top-dressing fertilizer in double-row (a) and single-row (b) planting arrangements.

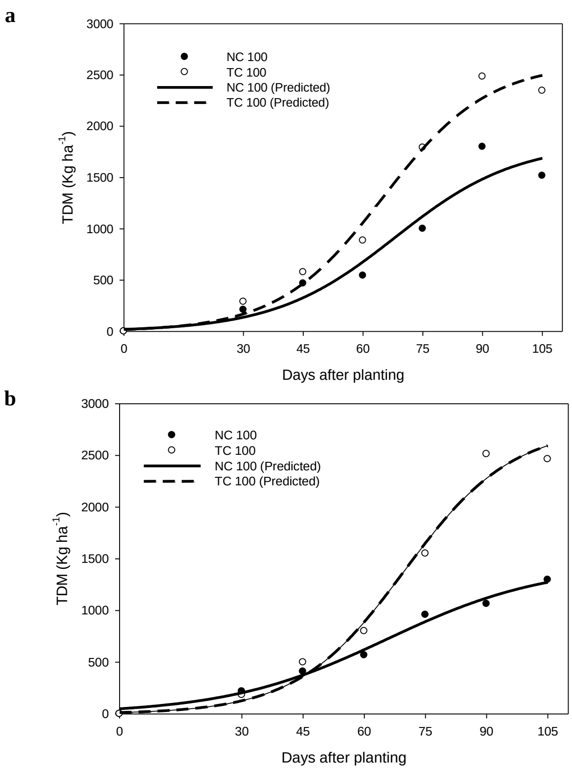

Figure 4. The trend of changes in TDM of NC100 and TC100 tobacco cultivars and the soil application of top-dressing fertilizer in double-row (a) and single-row (b) planting arrangements.

Results showed that double-row spacing of the TC100 cultivar recorded a higher TDM than the NC100 cultivar (Fig. 3a and 4a). The value of parameter m (the time of the maximum increase of TDM) was obtained in most of the cases in the soil application of top-dressing fertilizer (Data not shown).

3.3. Crop growth rate (CGR)

Fig. 5 and 6 present CGR changes in different treatments. At the beginning of the growth, the process of changes in the CGR was lower due to imperfect canopy closure and lower leaf surface to receive light efficiently. The decrease in the CGR at the end of the season can be related to the leaves aging and falling and the decrease in the photosynthesis level of the crop (Fig. 5 and 6).

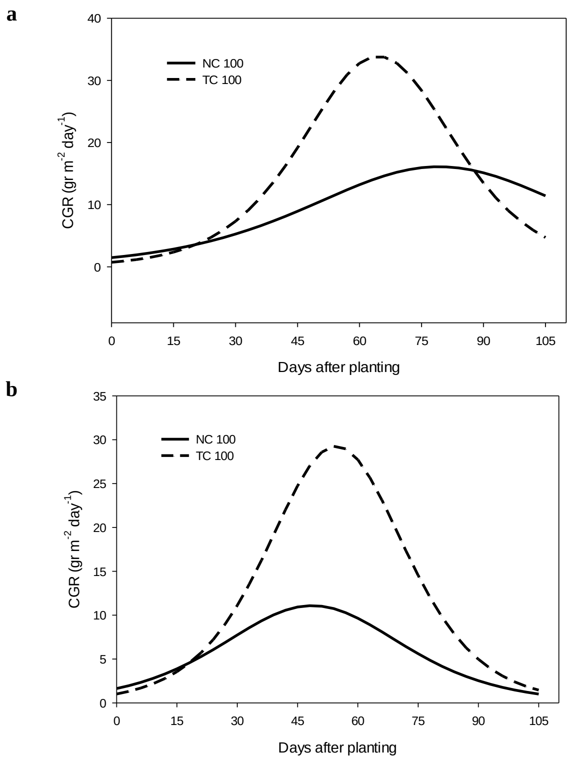

Figure 5. The trend of changes in CGR of NC100 and TC100 tobacco cultivars and the method of foliar application of top-dressing fertilizer in double-row (a) and single-row (b) planting arrangement

Fig. 5 shows the dynamics of CGR being characterized by a bell-shaped curve The highest CGR values were found in double-row spacing (16.2 $g.g^{-1}. day^{-1}$) in 70 DAP and (34.5 $g.g^{-1}. day^{-1}$) in 63 DAP in NC100 and TC100 cultivars, respectively (Fig. 4a) and, maximum values were obtained between treatments conventional single-row spacing, foliar application and TC100 cultivar (30.1 $g.g^{-1}.day^{-1}$) in 55 DAP and the NC100 cultivar (11.5 $g.g^{-1}.day^{-1}$) in 47 DAP (Fig. 5b). Also, the maximum value of CGR was obtained between treatments double-row spacing in the soil application, (27.8 g.g^{-1}.day^{-1}) in 69 DAP and (14.8 g.g^{-1}.day^{-1}) in 73 DAP in TC100 and NC100 cultivars, respectively (Fig. 6a). Also, the maximum value of CGR were obtained between treatments conventional single-row spacing in the soil application in TC100 cultivar (26.5 g.g^{-1}.day^{-1}) in 79 DAP and in NC100 cultivar (23.2 g.g^{-1}. day^{-1}) in 59 DAP (Fig. 6b).

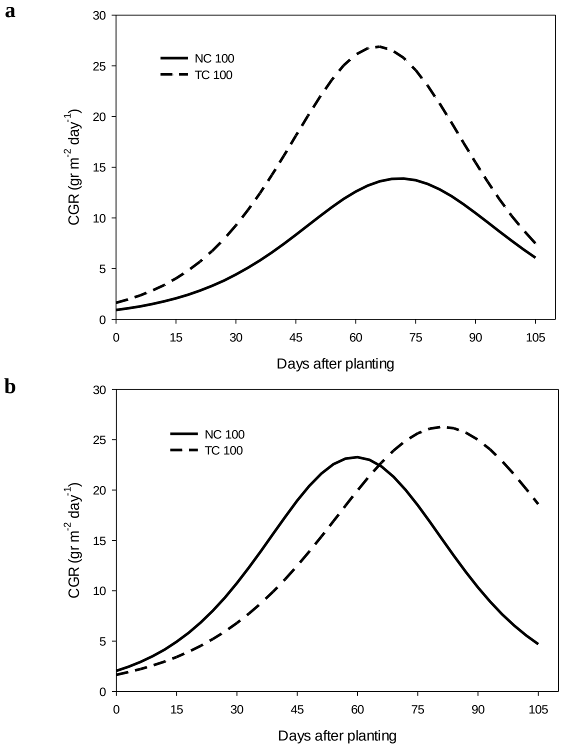

Figure 6. The trend of changes in the CGR of NC100 and TC100 tobacco cultivars and the method of soil fertilizer application in double-row (a) and single-row (b) planting arrangements.

3.4. Relative growth rate (RGR)

RGR expresses growth in terms of the rate of increase in size per unit of size (Sugar *et al*., 2017). Fig. 7 and 8 show the RGR changes trend in two cultivars of tobacco by planting arrangement in the method of foliar and soil applications. RGR declined with increasing age in all treatments. The dynamics of RGR exhibited an initial, relatively rapid increase at the beginning of the growing season, which represents the faster growth of the plant at the beginning of the growing season. The maximum value of the RGR (0.08 g. g^{-1}.day^{-1}) was obtained between the treatments of the double-row spacing in foliar application in TC100 cultivar (Fig. 7a). The trends show changes in the RGR of tobacco cultivars in two planting arrangement and either foliar (Fig. 7) or soil (Fig. 8) applications.

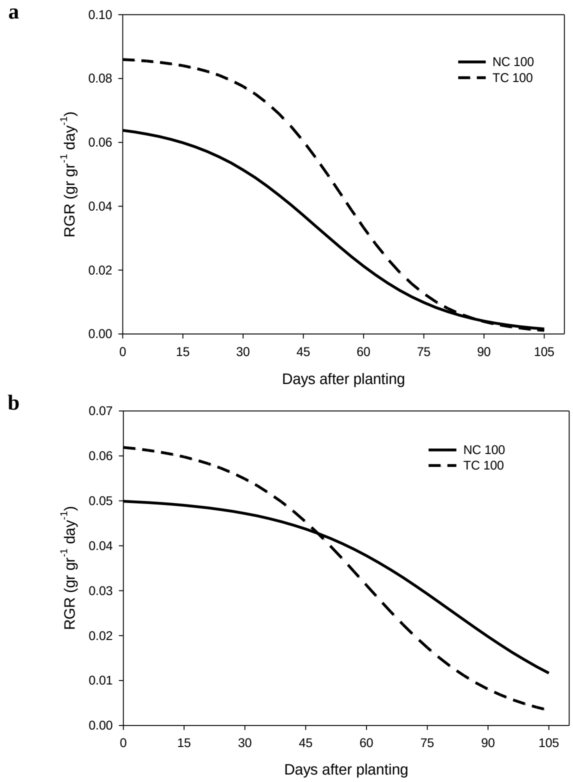

Figure 7. The trend of changes in the RGR of NC100 and TC100 tobacco cultivars and method of foliar application of top-dressing fertilizer double-row (a) and single-row (b) planting arrangement.

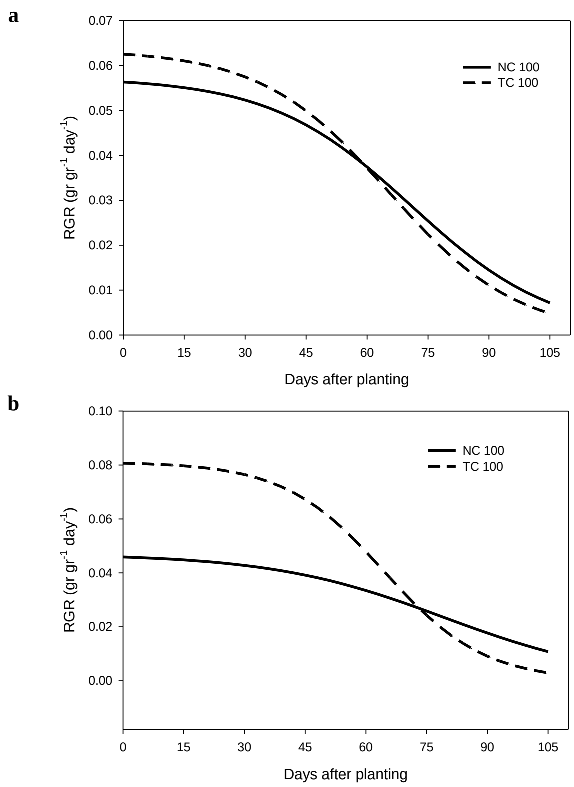

Figure 8. The trend of changes in the RGR of NC100 and TC100 tobacco cultivars and the method of soil fertilizer application in double-row (a) and single-row (b) planting arrangements.

Bullock et al. (1993) reported that an increase in the number of plants per unit area is considered an important factor to increase the RGR. In general, the amount of RGR at the beginning of the growing season, due to the better penetration of light into the canopy, less mutual shading of leaves and less respiration is higher. Then, with the progress of planting days and the development of leaves and increasing shading of the plant, the RGR decreases (Rameshjan *et al*., 2020). The lowest value of the RGR was obtained in the NC100 cultivar (0.04 g. g^{-1}.day^{-1}) in all treatments (Fig. 7 and 8). Malek et al. (2012) reported similar results in soybeans, where the RGR reduced during later growth stages (reproductive stage) which may be ascribed to excessive mutual shading as the LA was maximum during this period an enhanced number of old leaves could have lowered the photosynthetic efficiency in this experiment. RGR declined throughout the season and much of this decline would be attributed to an increase in self-shading among canopy leaves (Karimi and Siddique, 1991). It seems that the negative RGR values can be explained by an increase in the number of senesced leaves and leaf harvesting.

3.5. Green yield

Green yields were significantly affected by simple treatments and their interaction (Table 2). In the treatments of foliar fertilization, the double-row spacing of NC100 cultivars caused an increase of about 57% compared to conventional single-row cultivation. This value was only 0.5% in the double-row spacing of the TC100 cultivar (Fig. 9). It seems that the plant's physiological status and physical characteristics such as leaf shape, cuticle composition, the waxy structure of the leaf surface, leaf surface structure, the presence of leaf hairs, the phenological stage of the plant and the nutrients dynamics inside the plant are the main factors of the difference in leaf absorption by the cultivars (Henry *et al*., 2019). In cash crops like Virginia tobacco, this improvement in yield gives a prominent increase in net return to the farmers (Hema *et al*., 2021). In the soil fertilization treatments, the highest green yields were obtained from the TC100 cultivars in double-row spacing, and the lowest green yields were obtained from the NC100 cultivars in single-row spacing (Fig. 9).

Table 2. Variance analysis of green yield of tobacco

Source of Variation	df	Green leaf yield
Replication	2	45037
Method of fertilizer (A)	1	74731824**
Error (a)	2	104488
Cultivar (B)	1	324870416**
Error b	2	52279
A×B	1	24140204**
Planting arrangement (C)	1	1297569204**
A×C	1	335253750**
B×C	1	2114640**
A×B×C	1	121977468**
Error	12	142002
C.V. (%)		2.1

** and *significant at 1 and 5 %, respectively; ns non-significant

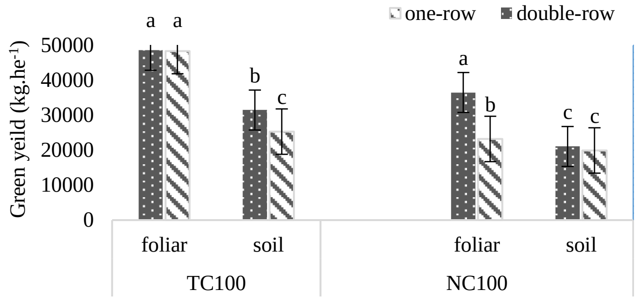

Figure 9. Effect of nutrition management and planting arrangement on green yield of two tobacco cultivars. The values followed by the same letter did not differ significantly (*P*<0.05) according to the LSD test.

4. Conclusion

The findings of this study illustrated that double-row spacing in the TC100 cultivar along with foliar spraying of N and K fertilizers leads to remarkable improvements in the tobacco growth indices and yield. As conventional single-row spacing is common practice in most regions of Iran, therefore, modern double-row spacing and foliar spraying of N and K fertilizer could be recommended.

Conflict of Interests

The authors declare no conflict of interest.

Ethics approval and consent to participate

No human or animals were used in the present research.

Consent for publications

All authors read and approved the final manuscript for publication.

Availability of data and material

All the data are embedded in the manuscript.

Authors' contributions

M.R. carried out all the experiments and she helped in preparing the manuscript, H.P., MA.E. and R.A. designed the project, analyzed the data, and wrote the manuscript and Y.Y. contributed to the supply of laboratory equipment.

Informed Consent

The authors declare not to use any patients in this research.

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