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Reaction of Tobacco to Plant Density Treatments at Three Different Dates of Transplanting

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Abstract

In this study, the effects of nine different transplanting date and plant density combinations on yield, quality, income, reducing sugar, and nicotine content of sun-cured tobacco (*Nicotiana tabacum* L.) were investigated to provide reference for optimization and improvement of transplanting date and plant density norms. Different combinations were created based on regional transplanting dates and plant densities. Transplanting dates were determined as 15 days before and after the reference transplanting date of May 27th, 2023. Plant densities were established using combinations of 30-40-50 cm row spacing and 8-12-16 cm intra-row spacing, being below and above the regional reference density of 40×12 cm. The experiment was conducted using a randomized plots design with split-split plots in Samsun ecological conditions. For all parameters examined, decreases following delayed transplanting schedule were observed at rates of 13.0% for yield, 14.7% for quality grade index, 22.6% for gross income, 32.8% for reducing sugars, and 35.7% for nicotine. Higher plant density led to increases in all parameters except nicotine, which increased with decreasing density. With decreasing plant density, yield decreased by 23.9%, quality grade index by 18.5%, gross income by 34.7%, and reducing sugars by 79.4%, while nicotine increased by 93.4%. The study demonstrated that advancing transplanting dates and increasing plant density presents a significant opportunity for improving yield and quality performance. Results should be supported with appropriate field equipment while monitoring water and nutrient use efficiency. Therefore, transplanting should be completed in May, and density should be increased to 250,000 pph or above where possible. Production targeting low nicotine content is possible with late transplanting and high plant density. Considering that increased plant density may be subject to more stress and could increase the risk of loss, this strategy should be carefully considered according to the expectations of the produced crop group.

Keywords: Abiotic Stress, Chemical Properties, Gross Income, Oriental, Row Spacing, Sun Cured

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INTRODUCTION

Tobacco (*Nicotiana tabacum* L.), which is cultivated in many tropical and subtropical regions of the world today, is an herbaceous plant from the Solanaceae family with annual or perennial species (Srinivasa et al., 2016). What distinguishes tobacco from most agricultural products is that its economically important part is the leaf rather than the seed. While tobacco can adapt to a wide range of conditions, it is highly sensitive to environmental factors. The ecological foundation for the development of high-quality tobacco leaves is the natural environment (Tang et al., 2020). Tobacco also has an extraordinary ability to compensate for early season losses. A 10-15% early season loss can be fully compensated, while a 50% surviving plantation can account for 80% of the total yield (Wilkinson et al., 2008). Transplanting date and fertilizer management, which affect the biophysical and chemical properties of tobacco vegetation, are direct sources of yield and quality differences (Svotwa et al., 2014; Regassa & Chandravanshi, 2016). Generally, while the expansion of plants' living space enables them to easily meet their needs such as nutrients, light, and water, it does not always increase the yield and income obtained per unit area (Kinay, 2023).

Abiotic stress factors are significant constraints that pose serious threats to agricultural production. Most studies aimed at developing plants resistant to abiotic stress have been conducted on model plants such as Arabidopsis, tobacco, and rice. However, despite numerous studies examining the underlying mechanism, plants' reactive responses to stressors have not been fully understood (Reguera et al., 2012; Esmaeili et al., 2022). This situation has revealed the necessity of reviewing appropriate plant density per unit area and transplanting/sowing calendar in recent years. Determining the optimal transplanting density and timing in regions can be the most important factor for maximum yield (Kharazmi et al., 2014). Continuous adjustments in plant density are among the most important practices necessary for increasing productivity (Okumura et al., 2014). Changes in transplanting design enhance the plant's ability to compete with factors such as higher intraspecific competition, unfavorable weather conditions, or low soil fertility (Testa et al., 2016).

It is possible to envision a high-density tobacco production model where income is generated from multiple sources, with biomass waste being used as raw material for protein, lutein, solanesol, biomethane production, as well as for producing medicines, vaccines, and industrial enzymes (Reynolds et al., 2022). The primary goal of increasing plant density is to enhance productivity in terms of grain or biomass, thus making the crop system more efficient and competitive per unit area. Plants placed at more regular intervals actually compete minimally for main growth factors. Among these factors, light is the most affected, followed by nutrients and water (Testa et al., 2016). In sun-cured conditions with K-326 tobacco variety, among seven different transplanting densities, 110×38 cm (23,923 pph) gave the highest values for dry leaf yield, while 90×40 cm (27,778 pph) norms provided the best quality and chemical content (Vural & Ekren, 2022). Another study conducted with Coker-347 virginia variety in 12 different transplanting density combinations reported that 14,285 pph was necessary for high yield and quality (Kharazmi et al., 2014). For Basma type tobacco, early transplanting advantage was mentioned, with the highest income reported at 40×8 cm (312,500 pph) transplanting conditions (Kinay, 2023). In a study on nitrogen uptake per decare of flue-cured virginia tobacco at varying plant densities, the best yield and quality were achieved at 16,650 pph conditions (Chao et al., 2021). Parallel to the increase in plant numbers per unit area, nutritional supplements like nitrogen need to be increased as well. Furthermore, while increasing plant density per unit area particularly decreases nicotine content (Henry et al., 2019), it leads to an increase in reducing sugar content. As row and intra-row spacing increases, plants can benefit more from daylight, leaf numbers increase, and leaves with thicker tissue (heavy bodied) can be obtained. In the opposite case, fewer leaves with thin tissue (light bodied) are formed (Mantesa et al., 2019).

Plant density per unit area directly affects agricultural crop yield and quality. While heterogeneous environmental conditions, even specific to countries and regions, are the main factors governing transplanting date, reactive differences can occur according to plant density and genotype. In any new ecology, the first step of the production process for a new type/variety is determining the correct transplanting date and density, which is of critical importance. Therefore, in addition to being a model plant, due to constraints in production conditions, this study aimed to determine the response to changes in transplanting date and plant density of oriental type tobacco, known as a stress plant, and to identify optimal levels.

MATERIALS AND METHODS

Plant material

As plant material, the registered *Nicotiana tabacum* L. var. OZE-S2 variety, known for its characteristic curing color and aroma and highly favored by producers, was used. Specifically, among oriental tobaccos, this variety is characterized by tall stature (142-150 cm), medium leaf density (34-36 leaves plant^{-1}), narrow (slightly) elliptical leaf shape, late maturity, light pink flower color, 11-15 cm leaf width, 24-28 cm leaf length, high yield and efficiency, and balanced nicotine/sugar ratio (Kinay & Kurt, 2022).

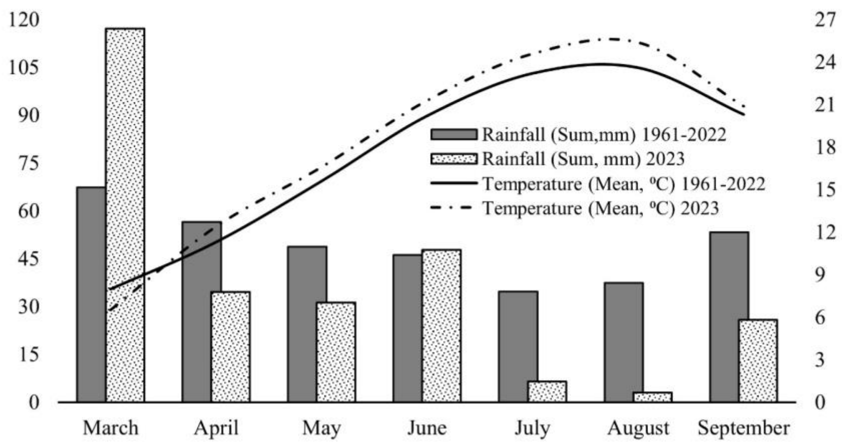

Figure 1. Meteorological data for experimental year and long-term averages.

Growth conditions

The research was conducted under rainfed farmer conditions in Samsun province during 2023, at coordinates 41º34'13"N, 35º51'53"E and an altitude of 21 meters. The average temperature during the vegetation period was similar to long-term averages. In the production season, which was drier compared to long-term averages, drought effect was most severe particularly during the flowering period. Excessive rainfall during the seedling period compared to long-term averages caused a delay in the production season. Total precipitation during the vegetation season decreased by 22.7%, reaching 266.4 mm (Fig. 1). The field, with its sandy-loam soil structure, had suitable texture for tobacco cultivation and slightly alkaline character (pH: 7.82). It was non-saline (EC: 765 dS cm^{-1}), calcareous ($CaCO_3$: 9.3%), and low in organic matter content (1.45%). P_2O_5 (32 kg da^{-1}), Mg (472 mg kg^{-1}), and Fe (6.27 mg kg^{-1}) contents were high. While Cu (1.76 mg kg^{-1}) and Zn (1.26 mg kg^{-1}) were at sufficient levels, K_2O (109.8 kg da^{-1}), Mn (8.01 mg kg^{-1}), and B (0.18 mg kg^{-1}) contents were low.

Seedlings were grown in foam trays with peat medium in a float system, where 20.10.10 NPK+micro compound (0.4-0.4-0.4 Fe-Mn-Zn) was added to pool water at a rate of 500 g ton^{-1}. Seedlings that reached 4-6 leaves, 12-15 cm height, and became hardened were transplanted to prepared plots. Prior to transplanting, 40-40-60 kg ha^{-1} NPK was applied to plots consisting of four rows of four meters length, followed by 30 kg ha^{-1} N application after the first hoeing. Throughout the vegetation period, maintenance operations such as hoeing, bottom leaf removal, earthing up, and disease-pest control were performed (Kurt, 2023).

Experimental procedures

The compared treatments were a factorial combination of different transplanting dates (DT), row spacing (RS), and intra-row spacing (IS) (Table 1). Transplanting were conducted on May 12th (DT-1), May 27th (DT-2), and June 12th (DT-3) at nine different transplanting densities. RS distances were 30 cm (RS-1), 40 cm (RS-2), 50 cm (RS-3), and IS distances were 8 cm (IS-1), 12 cm (IS-2), and 16 cm (IS-3). Matured leaves were harvested in three priming, wilted in shade for one day, and then sun-cured (Fig. 2). After complete drying, leaf tobacco was weighed, standardized to 15% moisture content, yields (YLD) were calculated, and organoleptic observations (quality grade index, QGI) were performed by tobacco experts according to the American Grading method. Gross income (GRI), calculated by yield×price, was determined based on grade yield. Three different dollar-based prices were established according to whether the grade yield was less than 55, between 55-60, or greater than 60 (2.64 \$ kg^{-1}<55%<2.91 \$ kg^{-1}<60%<3.17 \$ kg^{-1}) (Kurt, 2023). Samples for chemical content analysis were taken from weighed tobacco and ground at zero moisture. Nicotine (Coresta, 2017; NIC) and reducing sugar ratios (Coresta, 2010; RES) of the samples were analyzed using the continuous flow analysis method at Öz-Ege Tobacco Inc. analysis laboratory.

Table 1. Treatment combinations and plant densities per hectare

Row spacing (RS, cm)	Intra-row spacing (IS, cm)	Combinations (RS × IS)	Code (Dn_x)	Plant per hectare (pph)
30	8	30 × 8	Dn1	416,667
	12	30 × 12	Dn3	277,778
	16	30 × 16	Dn5	208,333
40	8	40 × 8	Dn2	312,500
	12	40 × 12*	Dn6	208,333
	16	40 × 16	Dn8	156,250
50	8	50 × 8	Dn4	250,000
	12	50 × 12	Dn7	166,667
	16	50 × 16	Dn9	125,000

*reference ranges

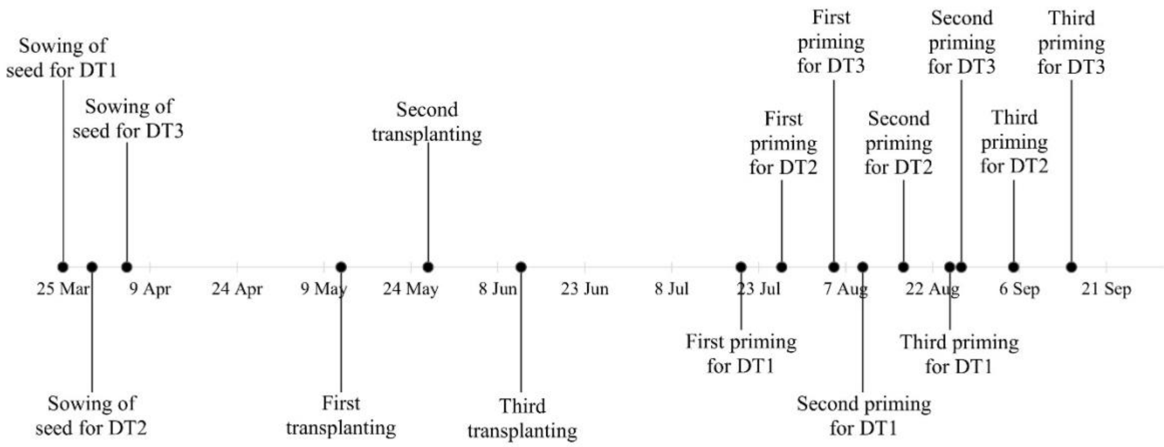

Figure 2. Tobacco sowing, transplanting and priming dates. DT; Date of transplanting.

Statictical analysis

The study was conducted in three replications according to a randomized plots design with split-split plots, where intra-row spacing, which required more precise examination, was assigned to sub-sub plots, row spacing to sub-plots, and transplanting date to main plots. Arcsine transformation was applied to quality grade index data. Data were subjected to analysis of variance (ANOVA) using JMP 13.0 software, and differences between means were grouped using Tukey's HSD multiple comparison test (Kurt, 2023).

RESULTS

Effect of transplanting date on tobacco characteristics

Transplanting 15 days before (May 12th; DT-1) and 15 days after (June 12th; DT-3) the reference date (May 27th; DT-2) significantly affected ($p<0.01$) the yield, quality, income, and chemical composition of sun-cured tobacco (Table 2). In the study, the average yield was realized as 1887.47 kg ha^{-1}, with the highest yield of 2033.52 kg ha^{-1} obtained from the first transplanting date (DT-1), which had its final harvest on August 25th and formed a statistically different group. The decrease in yield following the delay in transplanting calendar (DT-3<DT-2<DT-1) was different for DT-2 and DT-3, despite being in the same statistical group. When evaluated compared to DT-1, yield losses of -8.5% in DT-2 and -13.0% in DT-3 were determined (Table 3, Fig. 3).

Similarly, in the QGI value with a trial average of 57%, each transplanting date was listed in a different group, and advancing transplanting dates caused a decline in quality (DT-3<DT-2<DT-1). Compared to DT-1, which had the highest QGI value, the decrease was -9.1% for DT-2 and -14.7% for DT-3. The GRI value, which averaged 5560.86 \$ ha^{-1} and was calculated from yield and QGI performance, thus maintained the same ranking parallel to YLD and QGI, with the highest value obtained in DT-1 and the lowest in DT-3. The decrease in income compared to DT-1 was calculated as -12.6% for DT-2 and -22.6% for DT-3 (Table 3, Fig. 4, 5).

Table 2. The ANOVA table for the significance of treatments and their interactions according to split-split plot design in randomized plots for sun cured tobacco characteristics.

Source	DF	Yield			Quality grade index			Gross income			Reducing Sugar			Nicotine			$Q_{0.05}$
		MS		SE	MS		SE	MS		SE	MS		SE	MS		SE	
Block	2	99444		-	116.8		-	97745		-	1.934		-	0.145		-	-
DT	2	489178	**	20.9	613.9	**	0.4	13800000	**	42.6	86.02	**	0.11	5.550	**	0.03	3.564
Error 1	4	11788.9		-	3.753		-	49075.90		-	0.345		-	0.019		-	-
RS	2	188648	**	13.8	271.4	**	0.8	4555266	**	47.6	26.23	**	0.06	1.074	**	0.02	2.668
DT×RS	4	16931.5	*	23.8	9.162	ns	1.3	63764.90	*	82.5	0.060	*	0.11	0.041	**	0.03	3.721
Error 2	12	5118.89		-	16.02		-	61228.90		-	0.115		-	0.007		-	-
IS	2	287663	**	23.7	45.59	*	0.6	3435026	**	64.7	64.51	**	0.11	4.806	**	0.03	2.444
DT×IS	4	16786.7	*	41.0	1.686	ns	1.1	306103.0	*	112.9	0.205	*	0.19	0.011	*	0.05	3.297
RS×IS	4	102811	**	41.0	57.60	**	1.1	1898125	**	112.1	0.114	*	0.19	0.056	*	0.05	3.297
DT×RS×IS	8	14712.0	*	71.1	1.900	*	1.2	137975.0	*	194.1	0.385	*	0.34	0.013	*	0.08	3.999
Error 3	36	15146.7		-	11.37		-	113070.0		-	0.338		-	0.021		-	-

(**), (*), ns: significant at 1%, 5%, and nonsignificant, respectively, DF: Degree freedom, DT: Date of Transplanting, RS: Row Spacing, IR: Intra-row Spacing, MS: Mean square, SE: Standart error, Q: Q value for Tukey's HSD (α: 0.05).

Although varying proportionally, the negative effect of advancing transplanting calendar can also be mentioned in chemical composition. In RES values (mean 8.06%), when DT-1 was taken as the base, there was a decrease of -27.8% in DT-2 and -32.8% in DT-3. As another parameter, NIC was negatively affected by delayed transplanting, with a particularly severe decrease of -35.7% in DT-3 compared to DT-1. The most striking effect of changes in transplanting dates was observed in NIC amounts (Table 3, Fig. 6, 7).

Effect of plant density on tobacco characteristics

Nine different transplanting densities significantly affected all examined parameters. This significance was at $p<0.01$ level for all parameters except for the effect of IS on QGI ($p<0.05$) (Table 2). Factors were established at levels one below and one above the RS×IS reference values of 40×12 cm. As RS density increased, yield increased as well. The yield, which was 1813.56 kg ha^{-1} in RS-3, increased by 5.7% in RS-2 and 9.1% in RS-1 with increasing density, reaching 1978.18 kg ha^{-1}. Similarly, as transition occurred from RS-3 to RS-1, QGI increased, with the main increase occurring in RS-1. Compared to RS-3, there was a clear quality increase of 11% in RS-1. This enabled product pricing to reach 3.17 \$ kg^{-1}. When combined with yield increase, GRI increased by 7.1% in RS-2 and 16% in RS-1 compared to RS-3 (Table 3, Fig. 3, 4, 5).

The most significant variation in the study with changing RS occurred in RES and NIC values. RES increased by 23.5% in RS-1 compared to RS-3, reaching 9.2%. Similar results were achieved at RS-3 and RS-2 levels, while RES increased significantly when transitioning to RS-1. NIC, one of the chemical structures with opposite characteristics to RES, showed a contrary response to increasing RS levels, with NIC increasing at wider spacing. A linear increase of 13% in RS-2 and 21.7% in RS-3 was observed compared to RS-1. NIC reached from 1.84% to 2.24% as average values under wider RS conditions. Changing RS conditions caused significant changes in all parameters, with the widest variation detected in chemical structure (Table 3, Fig. 6, 7).

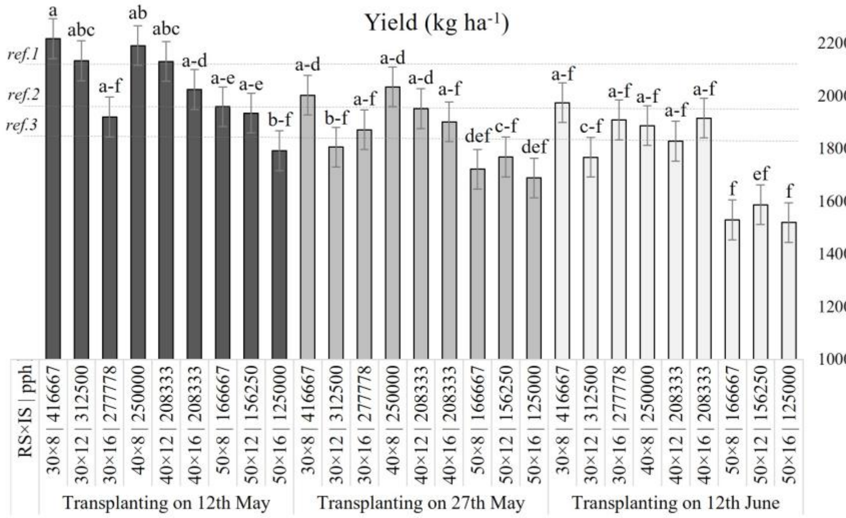

Figure 3. Effect of transplanting date and plant density on tobacco yield.RS×IS: Interaction of row spacing with intra-row spacing. pph: Plant per hectare. ref.1: Reference range (40×12 cm) for first date of transplating. ref.2: Reference range (40×12 cm) for second date of transplating. ref.3: Reference range (40×12 cm) for third date of transplating. Levels not connetted by same letter are significantly different.

The response of all parameters to changes in IS, except for RES, was in the same direction as in RS. Yield decreased with increasing IS distances, with the highest value of 2001.26 kg ha^{-1} obtained in IS-1. This decrease was -7.5% in IS-2 and -11.2% in IS-3 compared to IS-1. QGI showed a narrower variation under different IS conditions. This change occurred with a 4.2% increase in favor of IS-1. GRI value, derived from YLD and QGI, was also positively affected by increased density. Denser transplanting provided an income increase of 694.4 $ ha-1 (13.2%) in GRI. Contrary to the RS effect, RES values showed a favorable pattern for the reference IS value. While the lowest average RES was 6.7% in IS-3, it increased by 45.5% in IS-2 reaching the highest value of 9.75%. Subsequently, the ranking for RES was formed as IS-3<IS-1<IS-2. For NIC, parallel to RS response (IS-1<IS-2<IS-3), an increase occurred with decreasing density. NIC, which showed the strongest response to changing IS conditions, increased from 1.57% in IS-1 to 2.24% in IS-2, showing a 42.7% increase. Under IS-3 conditions, NIC was measured as 2.35%, showing almost a half-fold increase (49.7%) compared to IS-1. Under different IS conditions, variation was observed in all parameters to varying degrees, but the most significant response was detected in chemical structure (Table 3).

Effect of transplanting date and plant density interactions on tobacco characteristics

All parameters except QGI were affected by two-way and three-way interactions of factors at different significance levels ($p<0.01$ or $p<0.05$), while QGI showed significant variation only in RS×IS and DT×RS×IS interactions (Table 2).

Generally, it is observed that factors carried their individual effects into their interactions. The interaction of transplanting date (DT) with row spacing (RS) or intra-row spacing (IS) had similar effects on parameters. In DT×RS interaction, yield reached its highest value in DT-1×RS-1 interaction. While the trial average for YLD was 1887.47 kg ha^{-1}, under DT-1×RS-1 conditions, it was 2089.22 kg ha^{-1}, 10.7% higher than the average. Compared to the lowest value (DT-3×RS-3; 1645 kg ha^{-1}), there was 23.5% higher productivity. Although QGI was not affected by DT×RS, it caused different prices to meet with YLD, thus GRI was affected by this interaction. The highest GRI was calculated from DT-1×RS-1 and DT-1×RS-2 interactions as 6622.83 $ ha^{-1} and 6383.94 $ ha^{-1}, respectively. A significant difference of 2221.18 $ ha^{-1} occurred with the interaction that created the lowest GRI value, which was identified as a substantial income difference of 50.5%. Similarly in RES data, the highest RES was obtained from DT-1×RS-1 interaction. The lowest RES was obtained from DT-3×RS-3 interaction as 6.17%. A significant difference of 83.8% occurred between DT-1×RS-1 and DT-3×RS-3 (Table 2).

In DT×IS interaction, unlike individual effects, a wider variation than DT×RS interaction was detected in all parameters except QGI. Parallel to previous findings, YLD, QGI, and GRI parameters showed higher performance under early-dense intra-row conditions. This difference was 32.6% in YLD, 22.4% in QGI, and 51.7% in GRI. Under early transplanting (DT-1) conditions, RES at 12 cm (IS-2) and NIC at 16 cm (IS-3) intra-row spacing showed increases of 116.6% and 145%, respectively, compared to the lowest values. Under these conditions, RES content was determined as lowest at 5.47% (DT-3×IS-3), highest at 11.85% (DT-1×IS-2), and NIC content was lowest at 1.11% (DT-3×IS-1) and highest at 2.72% (DT-1×IS-3) (Table 2).

In the interaction of row spacing and intra-row spacing (RS×IS), following decreasing plant density, YLD decreased by 23.9%, QGI by 18.5%, GRI by 34.7%, and RES by 79.4%, while NIC, contrary to all others, increased by 93.4%. The highest YLD value was obtained from RS-1×IS-1 interaction with 2064.51 kg ha^{-1}, with RS-1×IS-3 and RS-3×IS-1 falling in the same statistical group. In QGI and GRI, the highest productivity was achieved at 30 cm row spacing (RS-1) conditions with any intra-row spacing. In other words, the positive effect of dense transplanting in early calendar is clear. RES showed high performance (10.89%) at 12 cm intra-row spacing (RS-1×IS-2) under early transplanting conditions, while for NIC, as an inverse structure, the maximum value was determined as 2.63% in RS-3×IS-3 interaction (Table 2).

Table 3. Effect of plant density treatments at three different date of transplanting on sun cured tobacco

Sources	DT	RS (cm)	IS (cm)	Yield (kg ha^{-1})		Quality Index (%)	Grade	Gross Income ($ ha^{-1})		Reducing Sugars (%)		Nicotine (%)	
Date of Transplanting (DT)	12th May			2033.52	a	64.10	a	6300.64	a	10.11	a	2.41	a
	27th May			1860.50	b	58.26	b	5506.85	b	7.30	b	2.20	b
	12th June			1768.40	b	54.66	c	4875.11	c	6.79	b	1.55	c
Row Spacing (RS)		30		1978.18	a	61.58	a	5967.20	a	9.20	a	1.84	c
		40		1870.68	b	59.97	a	5569.57	b	7.54	b	2.08	b
		50		1813.56	c	55.47	b	5145.84	c	7.45	b	2.24	a
DT × RS	12th May	30		2089.22	a	67.62		6622.83	a	11.34	a	2.15	c
		40		2030.68	ab	65.43		6383.94	a	9.50	b	2.44	b
		50		1980.66	abc	59.26		5895.16	bc	9.48	b	2.65	a
	27th May	30		1941.78	bcd	60.36		5916.17	b	8.40	c	1.99	d
		40		1824.92	def	59.05		5463.66	cd	6.78	d	2.21	c
		50		1814.79	ef	55.37		5140.71	de	6.71	de	2.41	b
	12th June	30		1903.54	cde	56.76		5362.58	d	7.87	c	1.39	f
		40		1756.43	fg	55.45		4861.11	e	6.34	de	1.60	e
		50		1645.22	g	51.77		4401.65	f	6.17	e	1.65	e
Intra-row Spacing (IS)			8	2001.26	a	59.93	a	5955.23	a	7.75	b	1.57	c
			12	1861.28	b	59.57	ab	5466.54	b	9.75	a	2.24	b
			16	1799.87	b	57.52	b	5260.83	b	6.70	c	2.35	a
DT × IS	12th May		8	2180.37	a	65.40		6911.76	a	9.62	b	1.92	c
			12	1967.70	b	64.85		6067.83	b	11.85	a	2.61	ab
			16	1952.49	b	62.04		5922.34	b	8.85	bc	2.72	a
	27th May		8	1947.46	bc	58.99		5829.77	bc	7.09	d	1.69	d
			12	1831.51	cd	58.73		5386.61	cd	9.01	bc	2.40	b
			16	1802.52	d	57.05		5304.17	d	5.79	ef	2.52	ab
	12th June		8	1875.97	bcd	55.39		5124.17	d	6.54	de	1.11	e
			12	1784.63	d	55.13		4945.19	de	8.38	c	1.70	cd
			16	1644.60	e	53.45		4555.98	e	5.47	f	1.83	cd
RS × IS		30	8	2064.51	a	60.79	a	6133.81	a	8.82	b	1.36	f
			12	1900.07	abc	61.97	a	5740.44	a	10.89	a	2.05	d
			16	1969.97	a	61.97	a	6027.35	a	7.90	c	2.11	cd
		40	8	1901.89	abc	59.50	ab	5665.53	ab	7.36	c	1.67	e
			12	1947.06	ab	62.13	a	5841.09	a	9.12	b	2.25	bcd
			16	1763.09	bcd	58.29	ab	5202.09	bc	6.14	d	2.32	bc
		50	8	2037.39	a	59.50	ab	6066.37	a	7.07	c	1.68	e
			12	1736.72	cd	54.62	bc	4818.11	cd	9.22	b	2.40	b
			16	1666.56	d	52.28	c	4553.05	d	6.07	d	2.63	a
DT × RS × IS	12th May	30	8	2217.00	a	66.55	ab	7027.89	a	10.83	bc	1.58	ijk
			12	1920.00	a-f	68.16	a	6086.40	a-d	13.26	a	2.40	b-e
			16	2130.67	abc	68.13	a	6754.21	ab	9.92	b-e	2.46	b-e
		40	8	2133.13	abc	64.83	abc	6762.03	ab	9.33	b-f	2.03	e-i
			12	2024.10	a-d	68.28	a	6416.40	abc	11.14	b	2.63	a-d
			16	1934.80	a-e	63.17	a-d	5973.38	a-d	8.02	e-i	2.67	abc
		50	8	2190.97	ab	64.83	abc	6945.36	a	8.69	d-g	2.16	d-h
			12	1959.00	a-e	58.12	a-f	5700.69	b-f	11.15	b	2.78	ab
			16	1792.00	b-f	54.83	c-f	5039.43	d-h	8.61	d-h	3.02	a
	27th May	30	8	2002.63	a-d	59.71	a-f	5985.44	a-d	7.85	f-j	1.48	j-m
			12	1871.33	a-f	60.68	a-e	5753.51	b-e	10.29	bcd	2.22	c-g
			16	1951.37	a-e	60.69	a-e	6009.58	a-d	7.05	g-k	2.26	c-f
		40	8	1805.53	b-f	58.63	a-f	5406.59	c-g	6.69	i-l	1.76	g-j
			12	1901.37	a-f	60.85	a-e	5690.15	b-f	8.23	e-i	2.38	b-e
			16	1767.87	c-f	57.66	a-f	5294.25	d-g	5.43	klm	2.48	b-e
		50	8	2034.20	a-d	58.63	a-f	6097.28	a-d	6.74	h-l	1.82	f-j
			12	1721.83	def	54.67	c-f	4716.19	e-h	8.49	d-i	2.60	a-d
			16	1688.33	def	52.81	def	4608.67	fgh	4.89	lm	2.82	ab
	12th June	30	8	1973.90	a-e	56.11	b-f	5388.10	c-g	7.77	f-j	1.03	m
			12	1908.87	a-f	57.08	b-f	5381.40	c-g	9.13	c-f	1.54	jkl
			16	1827.87	a-f	57.09	b-f	5318.24	d-g	6.72	h-l	1.60	ijk
		40	8	1767.00	c-f	55.03	c-f	4827.96	e-h	6.07	j-m	1.23	klm
			12	1915.70	a-f	57.25	a-f	5416.72	c-g	8.00	f-i	1.75	hij
			16	1586.60	ef	54.06	c-f	4338.65	gh	4.97	lm	1.82	f-j
		50	8	1887.00	a-f	55.03	c-f	5156.46	d-g	5.77	klm	1.06	lm
			12	1529.33	f	51.07	ef	4037.44	h	8.01	f-i	1.82	f-j
			16	1519.33	f	49.21	f	4011.04	h	4.71	m	2.05	e-i

*Levels not connetted by same letter are significantly different. DT: Date of Transplanting, RS: Row Spacing, IR: Intra-row Spacing.

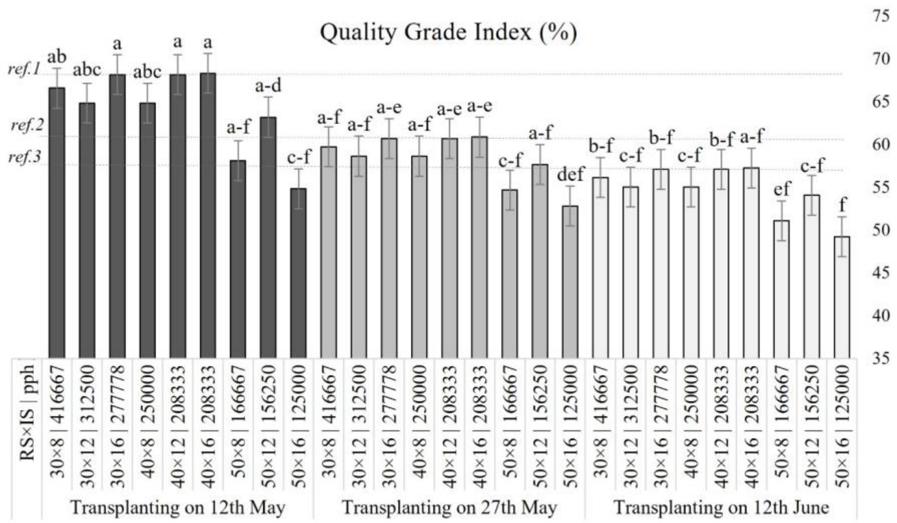

Figure 4. Effect of transplanting date and plant density on tobacco quality grade index. RS×IS: Interaction of row spacing with intra-row spacing. pph: Plant per hectare. ref.1: Reference range (40×12 cm) for first date of transplating. ref.2: Reference range (40×12 cm) for second date of transplating. ref.3: Reference range (40×12 cm) for third date of transplating. Levels not connetted by same letter are significantly different.

In the three-way interactions of transplanting date, row spacing, and intra-row spacing (DT×RS×IS), there is a decreasing but continuing significance in all parameters compared to individual or two-way interactions. The examined characteristics were affected by changing DT, RS, and IS conditions at $p<0.05$ level. Under the influence of all factors, YLD reached 2217.00 kg ha^{-1} under DT-1×RS-1×IS-1 conditions. The lowest YLD was realized as 1519.33 kg ha^{-1} under DT-3×RS-3×IS-3 conditions. Under three-way interaction conditions, the minimum and maximum values of QGI, GRI, RES, and NIC parameters were determined as 49.21-68.28%, 4011.04-7027.89 \$ ha^{-1}, 4.71-13.26%, and 1.03-3.02%, respectively. The response of YLD, QGI, GRI, and RES was similar at varying plant densities, with dense and early transplanting being identified as advantageous. However, NIC's response under the influence of all factors was different, with the best conditions being identified as early transplanting with low-density plant numbers. Under conditions of necessarily delayed transplanting calendar due to environmental conditions, low-density transplanting was found to be more advantageous in terms of maintaining NIC values (Table 2).

DISCUSSION

Date of transplanting

The study aimed to understand the response of oriental type tobacco, known as a stress plant due to production constraints in addition to being a model plant (Kurt et al., 2020), to changes in transplanting date and plant density. The common transplanting date in the region (DT-2) was taken as reference, and transplanting dates were established 15 days before and after. Plot transplanting's were conducted on May 12th, May 27th, and June 12th. The effects of changes in transplanting date manifested themselves in the priming schedule. The maturation processes for priming varied for plots planted on different dates. The number of days from transplanting to final priming was 104, 100, and 95 days according to DT order. First and second priming's were conducted on days 69-61-54 and 89-81-76, respectively. Delayed transplanting provided faster maturation compared to the first transplanting date: 8-15 days in first priming, 8-13 days in second, and 4-9 days in the final priming. As plants spend more time in soil, dry matter production and consequently yield increases (Kinay, 2023). Increasing stress sources with delayed transplanting may prevent plants from adequately meeting their needs. Environmental effects push tobacco to maintain its performance through biochemical and morphological mechanisms, experiencing yield losses while forming defense (Senbayram et al., 2015). As a result of this mechanism, late-planted plants couldn't receive sufficient nutrients, and sugar ratios decreased parallel to the delay (Fig. 6). The advantageous results of early transplanting also suggest that they utilized pre-transplanting nutrients more effectively.

Late-planted tobacco leads to faster growth, accelerated flowering initiation, production of thinner leaves, accelerated aging, and reduced yield (Abebe, 2024). Decreases following delayed transplanting calendar were present in all examined parameters. These decreases occurred at rates of 13.0% for YLD, 14.7% for QGI, 22.6% for GRI, 32.8% for RES, and 35.7% for NIC (Table 3). Better results were achieved with early transplanting due to increased seedling survival rate, agronomic characteristics, leaf appearance quality, suitability of leaf chemical composition, and improvement of leaves' economic indices (Qian et al., 2013). In a study reporting that as transition occurred from early to late transplanting, with the effect of ecological variables, yield, income, A-grade tobacco ratio, reducing sugar, sugar/nicotine ratio, aroma quality, aroma amount, and total sensory quality score first increased and then decreased (Su et al., 2023), similar to our results (Fig. 7), nicotine was reported to decrease gradually.

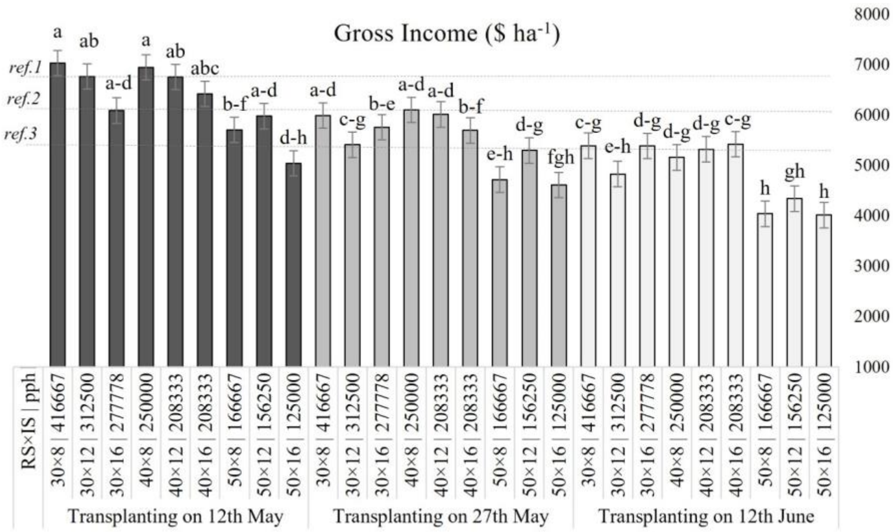

Figure 5. Effect of transplanting date and plant density on tobacco gross income. RS×IS: Interaction of row spacing with intra-row spacing. pph: Plant per hectare. ref.1: Reference range (40×12 cm) for first date of transplating. ref.2: Reference range (40×12 cm) for second date of transplating. ref.3: Reference range (40×12 cm) for third date of transplating. Levels not connetted by same letter are significantly different.

In a study conducted on Virginia type tobacco, it was reported that transplanting date could deviate at most two weeks from normal transplanting date, with significant yield losses occurring in transplanting conditions delayed by four and five weeks (Wilkinson et al., 2008). Similarly, in all parameters except reducing sugar and nicotine, the transplanting date data from May 27th and June 12th remained below the reference line (ref.1) of May 12th transplanting (Fig. 3-7). Nine different transplanting and harvesting time combinations were tested in Virginia tobacco (Peng et al., 2016), and almost exactly matching our study results, the highest performance in all examined parameters was obtained under conditions where transplanting date was advanced by 14 days. Parallel to our results, the delay in transplanting date from May 16th to June 13th resulted in decreases of 18.2% for YLD, 16.4% for QGI, 18.0% for GRI, 38.1% for RES, and 3.7% for NIC (Kinay, 2023).

In another multi-location study, yield loss averaged 8% per week starting from the third week after normal transplanting date, and in locations with very short growing seasons, yield loss increased from 8% to 36% when transplanting was delayed by four weeks (Wilkinson et al., 2008). It was demonstrated that insect damage increased and leaf yield decreased in delayed tobacco transplanting, while in early-planted ones, damage gradually decreased and leaf yield increased (Adebe, 2024). In early-planted flue-cured Virginia tobacco, polyphenol oxidase (PPO) activity remained at the lowest level, achieving better yellowing and curing quality. However, under delayed transplanting conditions, PPO reached the highest level, with browning occurring early, creating resistance to curing (Chao et al., 2017). According to the results, advancing the transplanting date by two weeks in the region will be one of the key practices in preventing yield, quality, and income losses.

Plant density

The contribution of increased plant numbers per unit area to yield has been reported in many studies. It has been clearly demonstrated that morphological adaptation of plants, especially crop-yield response, depends on plant density and row spacing (Testa et al., 2016). Decreasing plant population will affect leaf size and shape by increasing the stem/lamina ratio, which is undesirable for cigarette manufacturing companies. Additionally, increased nitrogen availability associated with decreasing plant numbers may cause an undesirable decrease in the alkaloid/sugar ratio in cured leaf (Wilkinson et al., 2008). Reduction in plants' living space pushes them to synthesize more sugar compounds and less nitrogenous compounds (Henry et al., 2019). Accordingly, in the research, nicotine and reducing sugar ratios changed inversely proportional to each other. Similar to our study results (Fig. 6, 7), a plant density study was conducted with K326 flue-cured Virginia tobacco variety at five levels between 14,200 pph and 20,500 pph, where RES increased from 18.6% to 21.3% with increasing density, while NIC decreased from 2.54% to 2.28% (Cao et al., 2019). In a study with sun-cured Virginia, RES increased and NIC decreased as plant density increased. As plant numbers per unit area increased, RES values moved from 5.6% to 9.7%, while NIC values moved from 1.9% to 1.1% (Vural & Ekren, 2022). In Basma type tobacco, the highest total income (8557.59 \$ ha^{-1}), indexed to yield and quality, was obtained at 40×8 cm transplanting density on May 16th (Kinay, 2023). In three different oriental type tobaccos, the positive effect of reducing transplanting distances on yield varied between 10-20%, with varying responses according to varieties, and nicotine was reported to increase with wider spacing (Bilalis et al., 2015). Researchers associated the yield decrease at wider spacing with reduced plant numbers per unit area. They attributed the decreased nicotine in narrow row spacing to high competition between roots where alkaloids are synthesized. Among twenty-eight tobacco varieties, including oriental types (Samsun and Xanthi), biomass yields under 75,000 pph conditions varied between 5.69-8.02 t ha^{-1}, and when plant density was increased from 40,000 pph to 125,000 pph, a significant increase in yield was detected (Reynolds et al., 2022).

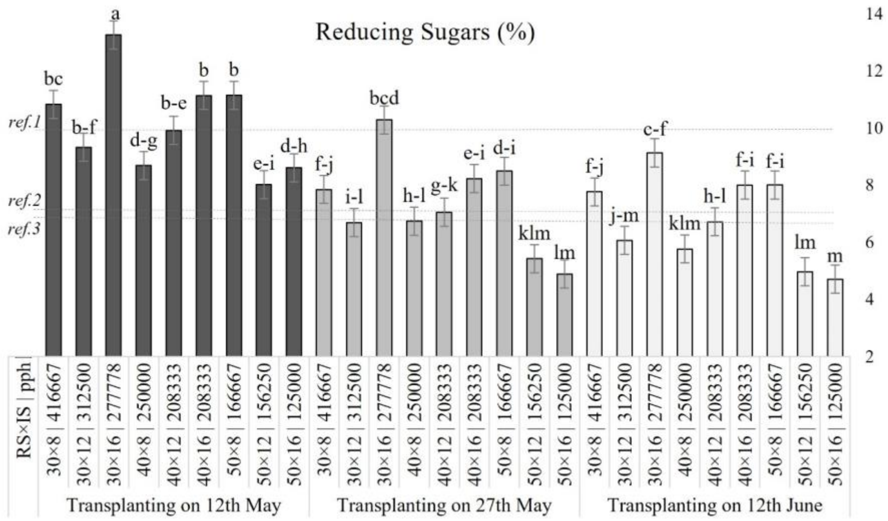

Figure 6. Effect of transplanting date and plant density on tobacco reducing sugars. RS×IS: Interaction of row spacing with intra-row spacing. pph: Plant per hectare. ref.1: Reference range (40×12 cm) for first date of transplating. ref.2: Reference range (40×12 cm) for second date of transplating. ref.3: Reference range (40×12 cm) for third date of transplating. Levels not connetted by same letter are significantly different.

Similar to the cited literature, in our plant density study at different transplanting dates, the highest yield was obtained as 2217 kg ha^{-1} at 30×8 cm spacing where the highest plant density (416,667 pph) was achieved under early transplanting conditions (Table 3, Fig. 3). Actually, under high-density conditions, the crop faces a more challenging and restrictive situation, mainly arising from intraspecific competition (Testa et al., 2016). However, at higher density, yield clearly increased as more leaves were harvested per unit area (Table 3, Fig. 3).

High plant density led to increases in all parameters except nicotine. However, the applicability of high density needs to be carefully considered in terms of meeting production conditions and mechanization requirements. Additionally, as access to nutrients and water becomes more difficult with increasing plant density per unit area, yield might be negatively affected due to increased impact of stressors under limited production conditions. In one study (Blandino et al., 2008), it was reported that ear rot severity and mycotoxin contamination increased when density was increased from 6.5 plants m^{-2} to 8.5 plants m^{-2} (Testa et al., 2016).

Adoption of increased plant density in tobacco production will contribute to producer income and supplier's yield and quality levels. High plant density allows better shading of the soil surface. More shaded row spacing will not provide suitable conditions for weeds and will reduce competition with weeds (Turgut et al., 2005; Fanadzo et al., 2010), which will positively affect yield. On the other hand, high plant density negatively affects plant growth due to insufficient sunlight and carbohydrate assimilation (Ciampitti & Vyn, 2012; Timlin et al., 2014). However, if germplasm is resistant to high competition for light, nutrients, and water between plants, high plant density can provide more yield (Brekke et al., 2011; Berzsenyi & Tokatlidis, 2012; Djaman et al., 2022). As can be understood, there is a need to determine not only the density level but also row and intra-row spacing. Despite the positive contribution of high density to the examined parameters, creating an applicable pattern is essential. An increasing feeding program will be needed following increasing plant density. Otherwise, the same amount of nutrients will be diluted to more plants, leading to a decline in leaf greenness. While sensitivity to drought stress may increase as water evaporates from more plants at high density, the amount of water evaporating from bare soil areas will decrease. Thus, a two-way situation will emerge, where plant water use efficiency increases while requirements also increase. Yield decreasing with increasing plant density in extremely dry years had a parabolic relationship with plant density in dry, wet, and extremely wet years (Ren et al., 2016; Djaman et al., 2022). Therefore, in non-irrigated or less water-available areas, lower plant density is preferred compared to opposite conditions (Testa et al., 2016). In addition to water and nutrient conditions, considering the increased labor requirement and unfavorable structure for mechanization with increasing plant numbers, it is recommended to increase plant numbers to 250,000 pph level by keeping row spacing at 40 cm and reducing intra-row spacing to 8 cm. Researchers evaluating tobacco for other uses (Reynolds et al., 2022) emphasized the necessity of production at a density of at least 250,000 pph as a critical factor in reducing bio-products production costs and tobacco's capability for this.

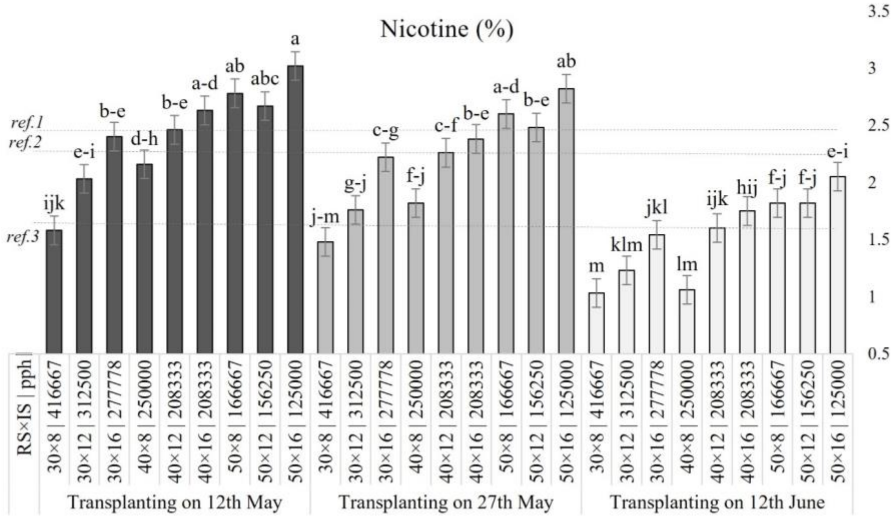

Figure 7. Effect of transplanting date and plant density on tobacco nicotine. RS×IS: Interaction of row spacing with intra-row spacing. pph: Plant per hectare. ref.1: Reference range (40×12 cm) for first date of transplating. ref.2: Reference range (40×12 cm) for second date of transplating. ref.3: Reference range (40×12 cm) for third date of transplating. Levels not connetted by same letter are significantly different.

CONCLUSION

While placing the plantation in optimal growing conditions, even if a high-density design is adopted, a balanced transplanting pattern should be considered. Thus, intraspecific competition will decrease, and it may contribute to yield even in low-density production. It will be possible to improve tobacco's physiological, morphological, agronomic, and technological characteristics. In the ecological conditions where the study was conducted, it was understood that plant density of 250,000 pph and above could provide significant yield increases. The limits of high-density conditions should be within the extent permitted by agricultural mechanization. Otherwise, individual plant performance will decrease, and stress conditions may become prominent. Healthy and high-density production can also prevent individual losses. Advancing transplanting date by two weeks will be key in preventing yield, quality, and income losses. The tobacco production season varied between 95-104 days (excluding seedbed). The advantage of denser transplanting in the early calendar is clear. Under necessarily delayed transplanting conditions, sparse transplanting is required for nicotine, while dense transplanting is necessary for other examined parameters. The performance of YLD, QGI, GRI, and RES is higher in early-period, dense row spacing conditions. NIC, however, increased with sparse transplanting in the early period. The decreasing nicotine value in dense transplanting can be kept in balance with the increase in total nicotine amount to be obtained from more plants per unit area.

Compliance with Ethical Standards

Peer Review

This article has been peer-reviewed by independent experts in the field using a double-blind review process.

Conflict of Interest

The author declares that there is no conflict of interest.

Author Contribution

The author solely conceived, designed, and conducted the study, analyzed the data, and wrote the manuscript.

Ethics Committee Approval

Ethical approval was not required for this study.

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Generative AI Statement

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Abbreviations

ANOVA, analysis of variance; DT, date of transplanting; GRI, gross income; IS, intra-row spacing; NIC, nicotine; pph, plants per hectare; PPO, polyphenol oxidase; QGI, quality grade index; RES, reducing sugar; RS, row spacing; YLD, yield.

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