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PERFORMANCE AND CORRELATIONS FOR AGRONOMIC AND MORPHOLOGICAL TRAITS OF FLUE-CURED TOBACCO (*N. TABACCUM* L.) UNDER CONTRASTING NITROGEN FERTILIZATION REGIMES

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Abstract

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In order to investigate the effect of nitrogen fertilization on agronomic and morphological traits of flue-cured tobacco, four flue-cured tobacco cultivars were grown at two contrasting nitrogen (N) rates (30 and 60 kg N ha^{-1}), at two Croatian locations (Kutjevo and Virovitica) with different soil types, for two years (2010 and 2011). The higher N fertilization rate resulted in significantly higher yields, value ha^{-1}, and area of the ninth leaf in 2010 while in 2011 between the two N rates differences were no significant. A significant cultivar x N fertilization rate interaction was not observed, and the traits evaluated were more influenced by year and location then by fertilization rate. Correlation analysis indicated that under different environmental conditions the pattern of correlations among important traits could be changed. The results suggest that 60 kg N ha^{-1} provided high yield of cured tobacco leaf without negative impact on its quality. The same N fertilization rate can also compensate the loss of nitrogen caused by leaching during the wet seasons.

Key words: flue-cured tobacco, nitrogen fertilization

Introduction

Nitrogen (N) fertilization of tobacco is crucial for achieving high yields of high-quality cured leaf. The optimal amount of applied nitrogen varies within a narrow window. Controlling the amount and timing of N availability during growth and ripening of tobacco, along with the ability to control soil moisture levels, has a greater impact on agronomic and smoking characteristics than any other nutrient variable or agrotechnological intervention (Collins and Hawks, 1993; Peedin, 1999; Marchetti et al., 2006). Experiments and observations have shown that the basic necessary amounts of N can vary from about 40 kg ha^{-1} to about 90 kg N ha^{-1}, depending upon soil type (Akehurst, 1981; Flowers, 1999; Peedin, 1999; Reed and Jones, 2002; Smith and Wood, 2005). On loamy sands, applications of supplemental N are often recommended to replace N lost due to leaching early in the growing season (Marchetti et al. 2006). For the climate and soils of northern Italy, Marchetti et al. (2006) found that in 1998 growing season 40 kg N ha^{-1} were sufficient to allow for optimal cured-leaf yields and to balance decreases in inorganic soil N. They also reported that the total amount of available soil N might have exceeded the N requirements for maximum production efficiency. On the other hand, Cristanini (2006) for agroecological conditions of north Italy recommended higher N fertilization rates ranging from 80 to 120 kg ha^{-1}.

Sisson et al. (1991) found that superior yield and quality of newer tobacco cultivars, in comparison to the older ones, was accompanied by improved nitrogen use efficiency. Same authors indicated the possibility of developing flue-cured tobacco cultivars adapted to soils with specific fertility levels and N supplies. Budimir et al. (2008) conducted a three-year

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field experiment to study yield, average price, and value ha^{-1} of Croatian flue-cured tobacco cultivars under two fertilization regimes (60 kg N, 35 kg P_2O_5, 210 kg K_2O ha^{-1}; and 40 kg N, 25 kg P_2O_5, 150 kg K_2O ha^{-1}) at two Croatian locations representing a silt loam soil and a sandy loam soil. The authors found significantly higher values for all studied traits in the sandy loam environment under the higher fertilization rate. Effects in the silt loam environment were non-significant. Differences between the two fertilization rates, averaged over soil types, were non-significant for all of the traits that were evaluated. The authors also reported significant location × fertilization rate and location × cultivar interactions for the studied traits. Detailed information regarding the effect of soil type and N fertilization on performance of modern Croatian flue-cured tobacco cultivars is still lacking. This study was performed in order to determine the differences in agronomic and morphological traits of four modern flue-cured tobacco cultivars at two N fertilization rates, and to determine the effect of soil type on performance of the same cultivars. In addition, phenotypic correlations among the studied traits were determined in order to find out the relationship between the traits, which form the yield and quality of the cured tobacco leaf at two N fertilization rates.

Materials and Methods

Three flue-cured tobacco cultivars widely grown in Croatia (DH17, DH27 and DH36) and NC55 (the most popular U.S. flue-cured cultivar in Europe), were chosen for this investigation. The cultivars were evaluated at two locations, Kutjevo and Virovitica, during 2010 and 2011. The soil at the Kutjevo location is a silty loam, heavier and cooler, with medium organic matter content. The soil at the Virovitica location is a sandy loam with poor organic matter content (Table 1).

Each year, tobacco transplants were produced hydroponically according to Boić et al. (1999). Transplanting was performed in May in plots with 100 cm row spacing and 50 cm plant spacing within rows. The experimental design in each environment was a randomized complete block design with four replications and a factorial combination of two N levels, 30 and 60 kg N ha^{-1}. Individual plots consisted of three 22-plant rows. Fertilization with phosphorus and potassium was performed before planting in quantities typical for commercial flue-cured tobacco production in Croatia (30 kg ha^{-1} P_2O_5 and 180 kg ha^{-1} K_2O). Fertilization with N was carried out using calcium nitrate as the source, with half of the specified amount incorporated in the soil prior to transplanting, and the remainder during the first cultivation (approximately ten days after transplanting). All other cultivation practices were those recommended for flue-cured tobacco production in Croatia.

Twenty plants of the middle row of each experimental plot were analyzed for the following morphological traits: plant height (cm), leaf number per plant, and area (cm^2) of the ninth leaf. Area of the ninth leaf was calculated by multiplying its length and width by the coefficient of 0.6345 according to Tso (1972). Leaves were harvested manually in four primings. Curing of harvested leaves was performed in bulk curing barns with heated air. After curing, yield (kg ha^{-1}) of each experimental plot was determined. Average price (€ kg^{-1}) was ascribed to the cured leaf of each plot by a tobacco cooperative agronomist. Total value (€ ha^{-1}) for each plot was calculated by multiplying yield by its respective price. Analyses of variance appropriate for the experimental design were performed for all measured characteristics using SAS Release 9.1 (SAS Institute, Cary, NC, USA). Cultivar, location, year, and fertilization rate were considered as fixed factors. Due to the year sum of squares comprising a high fraction of the total sum of squares for most traits, and because of significant observed interactions involving year, statistical analysis was performed for each year separately with all variables considered as fixed factors. Cultivar means at both N fertilization rates were compared using the Bonferroni-Dunn multiple comparison test (Dunn, 1961). Each year for both fertilization rates Pearson's correlation coefficients were determined among the all studied traits.

Results

During 2010, the total precipitation at Kutjevo (1258.8 mm) and at Virovitica (1022.8 mm) was greater than the multi-year average for Croatia (approximately 850 mm). In 2011, the total amounts of precipitation at Kutjevo and Virovitica were only 557.5 mm and 551.9 mm, respectively. The distribution of precipitation was similar at both locations dur-

Table 1
Results of the soil analysis at Kutjevo and Virovitica in 2010

Location	Soil type	pH		Al mg $100g^{-1}$		Humus	Hy
		H_2O	KCl	P_2O_5	K_2O	%	C mol kg^{-1}
Virovitica	Sandy loam	5.9	4.76	12.55	17.44	1.5	2.74
Kutjevo	Silty loam	5.48	4.32	25.8	26.45	2.01	4.55

ing 2011, with several hot and dry periods during the growing season between May and September.

Significant differences were detected between locations and cultivars for all measured traits during 2010 (data not shown). Significant differences were detected between N rates for yield and value ha^{-1}. A significant cultivar x N rate interaction was observed for leaf number, and significant cultivar x location interactions were observed for yield, price, value ha^{-1}, and leaf number. The location × N rate interaction was significant for price and leaf number. The location × cultivar × N rate interaction was significant only for yield. In 2011, significant differences were found between cultivars and locations for all studied traits. Differences between N rates were non-significant for all characteristics. The only significant interaction term was the cultivar × location interaction for price and value ha^{-1}.

In 2010, all traits except number of leaves had higher values at Virovitica than at Kutjevo (Table 2). In 2010, the Virovitica location produced approximately 800 kg ha^{-1} higher yield, approximately 0.20 € kg^{-1} higher price, and about 2200 € ha^{-1} higher value as compared to the Kutjevo location. Plants at the Virovitica location were on average 4 cm higher and produced leaves with approximately 150 cm^2 larger area. In 2011, however, all traits had significantly higher values at the Kutjevo location as compared to the Virovitica location. The Kutjevo location produced approximately 700 kg ha^{-1} higher yield, 0.10 € kg^{-1} higher prices, and about 1600 € ha^{-1} higher value as compared to the Virovitica location. Differences in morphological traits between the two locations were more pronounced in 2011, when plants at Kutjevo were about 19 cm higher and produced approximately three more leaves with increased area as compared to plants at the Virovitica location.

In 2010, all cultivars responded positively to increased N fertilization, with the 60 kg N ha^{-1} rate producing, on average, 217.8 kg ha^{-1} higher yields than the 30 kg N ha^{-1} rate (Table 3). Cultivar DH17 had the highest yield at both N fertilization rates with an average of 2801 kg ha^{-1}. The same cultivar had also the smallest average price (2.08 € kg^{-1}), while the highest price was observed for the U.S. cultivar, NC55 (2.30 € kg^{-1}). The average price across cultivars was not significantly affected by N rate, while the average value ha^{-1} was significantly higher at 60 kg N ha^{-1} than at 30 kg N ha^{-1}. Cultivars DH17 and NC55 had highest average value ha^{-1} (5871.69 and 5848.05 € ha^{-1}, respectively), although NC55 performed slightly better at the lower N rate and DH17 at the higher N rate. Differences for plant height and number of leaves were not significant between the two N fertilization rates (Table 4). The area of the ninth leaf was significantly greater at the higher N fertilization rate. Croatian cultivars DH17, DH27 and DH36, had simultaneously significantly greater plant heights and less leaves than NC55. Cultivar DH17 had the lowest leaf number, but the largest leaf area, which was approximately 100 cm^2 higher than that for the other three cultivars.

In 2011, differences between the two N fertilization rates were not significant for any trait under study (Tables 3 and 4). Cultivars DH36 produced the highest yields at either N rate, and DH17 produced cured leaf with a significantly lower price as compared to other three cultivars.

Cultivars DH27 and DH36 both produced higher values ha^{-1} than cultivar DH17. Cultivars DH27 and DH36 also had significantly higher plant heights than DH17 and NC55. Similar to 2010, NC55 had a significantly higher number of leaves than other three cultivars. The two cultivars with the smallest leaf number (DH17 and DH36) had the largest leaves (1050.24 and 1069.96 cm^2, respectively).

The Pearson's correlation coefficients among the studied traits at two nitrogen fertilization rates for 2010 and 2011 are presented in Tables 5 and 6. While among the agronomic traits yield, price and value positive correlations were determined for both fertilization rates and both years, among the other traits patterns of correlations differed between years and between N fertilization rates. In 2011, a higher number of trait pairs showed significant correlations than in 2010. This

Table 2
Average values for location and year for yield (kg ha^{-1}), price (€ kg^{-1}), value (€ ha^{-1}),plant height (cm), number of leaves and area of the ninth leaf (cm^2)

Year	Location	Yield	Price	Value	Plant height	Number of leaves	Area of the ninth leaf
2010	Kutjevo	2174	2.09	4530	79.72	20.16	538
	Virovitica	2966	2.33	6890	84.07	19.58	706
Significance		**	**	**	**	**	**
2011	Kutjevo	3746	1.76	6608	99.36	23.28	1030
	Virovitica	3024	1.65	5004	80.33	19.98	862
Significance		**	**	**	**	**	**

* - significant at P< 0.05; **- significant at P < 0.01; n.s. - not significant

case could be observed especially for correlations with the number of leaves at 30 kg N ha^{-1} and for correlations with plant height at 60 kg N ha^{-1}, which were in 2010 all not significant.

In 2010, stronger correlations among traits were observed at 30 kg N ha^{-1}, while in 2011 correlations among traits were stronger at 60 kg N ha^{-1}. In rainy 2010, correlation between yield and ninth leaf size was at both N fertilization levels strong and positive, correlation between yield and plant height was moderate and positive only at 30 kg N ha^{-1}, while between yield and leaf number correlation was moderate and negative only at 60 kg N ha^{-1}. In 2011, correlations between yield and plant height and between yield and leaf number were both strong and positive, while between the yield and ninth leaf size they were positive and moderate. The moderate negative correlation in 2010 between the ninth leaf size and number of leaves at 60 kg N ha^{-1} was in 2010 not significant and negligible.

Discussion

In the present study, the higher N fertilization rate resulted in significantly higher yield and value only during the year 2010. In this year, which was characterized by high precipitation levels, some N may have been lost due to leaching. In the 2010, the higher N fertilization rate may have compensated for N lost due to leaching. In such wet seasons, and on loamy sand soils, supplemental N applications are often suggested (Marchetti et al., 2006). Also in 2010, the values for all measured traits, except leaf number, were higher at Virovitica, which is characterized by deep sand soil that is poor in organic matter. On soils of this type, water can be

Table 3
Average values for yield (kg ha^{-1}), price (€ kg^{-1}), value (€ ha^{-1}) of the studied flue-cured tobacco cultivars for the years 2010 and 2011

Cultivar	2010			2011		
	Fertilization, kg N ha^{-1}		Average	Fertilization, kg N ha^{-1}		Average
	30	60		30	60	
			Yield, kg ha^{-1}			
DH17	2677	2926	2801 a#	3286	3312	3299 ab
DH27	2302	2557	2429 b	3378	3507	3442 ab
DH36	2431	2635	2533 b	3501	3571	3536 a
NC55	2436	2600	2517 b	3214	3316	3265 b
Average	2461	2679		3345	3426	
Significance	**			n.s.		
			Price, € kg^{-1}			
DH17	2.05	2.1	2.08 c	1.57	1.62	1.59 b
DH27	2.2	2.25	2.22 b	1.79	1.79	1.79 a
DH36	2.22	2.24	2.23 b	1.71	1.71	1.71 a
NC55	2.32	2.28	2.30 a	1.77	1.69	1.73 a
Average	2.2	2.22		1.71	1.7	
Significance	n.s.			n.s.		
			Value, € ha^{-1}			
DH17	5557	6187	5872 a	5141	5354	5248 b
DH27	5116	5766	5441 b	6122	6318	6220 a
DH36	5442	5914	5678 ab	6011	6143	6077 a
NC55	5711	5986	5848 a	5700	5657	5678 ab
Average	5456	5963		5743	5868	
Significance	**			n.s.		

* - significant at $P < 0.05$; **- significant at $P < 0.01$; n.s. - not significant
# - Average values followed by the same letter are not significantly different according to a Bonferroni- Dunn multiple comparison test ($P < 0.05$).

leached through the soil profile more quickly as compared to the heavier soils typical of the Kutjevo region. According to Cristanini (2006) the best soils for production of aromatic Virginia tobaccos are deep sandy soils poor in organic matter and nutrient content. Such soils serve only as a growing media, on which planned fertilization program can easily be implemented, especially if irrigation is provided. In the 2011, significantly higher trait values were observed at the Kutjevo location, an environment with a soil type, which seems to permit better drought tolerance due to higher water reserves accumulated in the soil. Although the price of flue-cured tobacco tends to decline with increased N fertilization rate due to prolonged ripening, (Sisson et al., 1991; Marchetti et al., 2006), such trend was not observed in our study except for the U.S. cultivar NC55. Differences between the two N rates were in 2011 non-significant, although the higher N rate (60 kg N ha^{-1}) resulted in slightly higher yield and value ha^{-1} of the cured leaf. In Croatia, flue-cured tobacco is usually fertilized with the N fertilization rate of about 45 kg ha^{-1} (Budimir et al., 2008). The results of the present study suggest that during wet seasons additional N up to 60 kg ha^{-1} could be applied as sidedress without negative effect on the cured leaf quality.

Cultivar × fertilization interactions were not significant in either 2010 or 2011 for the measured agronomic traits, indicating in a broad sense that none of the studied cultivars could be considered as being better adapted to the higher or lower N fertilization rates examined in this study. Cultivar × location interaction was in 2010 significant for yield, price, value and leaf number and in 2011 for yield and price. The location comprises environmental factors like precipitation, inclination, elevation, soil type and others. In Croatia, the lack of the typical tobacco arable land pushed the production towards

Table 4
Average values for plant height (cm), number of leaves and area of the ninth leaf (cm^2)of the studied flue-cured tobacco cultivars for the years 2010 and 2011

Cultivar	2010			2011		
	Fertilization, kg N ha^{-1}		Average	Fertilization, kg N ha^{-1}		Average
	30	60		30	60	
			Plant height, cm			
DH17	82.9	80	81.45 b	84.58	88.35	86.46 b
DH27	85.08	85.12	85.10 ab	93.04	93.38	93.21 a
DH36	88.63	85.92	87.27 a	93.81	93.04	93.43 a
NC55	71.85	75.67	73.76 c	84.89	87.68	86.28 b
Average	82.11	81.68		89.08	90.61	
Significance	n.s.			n.s.		
			Number of leaves			
DH17	18.73	18.32	18.53 d	19.94	20.15	20.04 c
DH27	20.38	20.08	20.23 b	22.19	21.96	22.08 b
DH36	19.38	19.07	19.23 c	21.15	20.63	20.89 c
NC55	21.03	21.97	21.50 a	23.19	23.85	23.52 a
Average	19.88	19.86		21.62	21.65	
Significance	n.s.			n.s.		
			Area of the ninth leaf, cm^2			
DH17	665	770	718 a	1032	1068	1050 a
DH27	506	628	567 b	822	884	853 b
DH36	554	659	606 b	1046	1094	1070 a
NC55	554	642	598 b	800	823	812 b
Average	570	675		925	967	
Significance	**			n.s.		

* - significant at $P < 0.05$; **- significant at $P < 0.01$; n.s. - not significant

\# - Average values followed by the same letter are not significantly different according to a Bonferroni- Dunn multiple comparison test ($P < 0.05$).

the heavier soils richer in organic matter, and for now, almost all tobacco is produced without irrigation. The performance of the cultivars observed over locations (data not shown) suggests that the three Croatian flue-cured tobacco cultivars in both experimental years at both examined locations reacted similarly in respect to the differences in soil type, Kutjevo- silt loam soil, Virovitica- sandy loam soil.

The results of the correlation analysis of the present study showed that both environmental factors (year) and nitrogen fertilization level influenced the pattern of correlations among studied traits. Correlations among the agronomic traits were in both experimental years positive at both N fertilization rates. Bukan et al. (2006) and Bukan et al. (2010) found positive correlations between yield and value and between price and value of the flue-cured tobacco earlier, but correlation between yield and price was in Bukan et al. (2006) positive and in Bukan et al. (2010) negative. Šmalcelj and Kozumplik (1980) also found negative correlation between yield and price, but for different flue-cured tobacco cultivars and cultural practices than used in the present study. Bowman and Sisson (2000) compared yield performance of U.S. flue-cured tobacco cultivars released in the early 1960s with cultivars in the late 1990s, and estimated a positive relative change of only 4.8% over the 36 years of breeding. According to these authors, relatively low rates of yield increase could be related to the U.S. Regional Minimum Standards Program for Flue-Cured Tobacco, which was initiated in 1964. This program has stringent quality requirements (nicotine and reducing sugar content, flavor of the tobacco smoke) that must be met before variety commercialization. For that reason, the correlation between yield and price of tobacco can sometimes be negative, especially if the price is considered as a quality index. Afzal Naz and Shafique (1979) showed that yield of tobacco can be predicted on the basis of leaf size, plant height,

Table 5
Pearson's correlation coefficients among the studied traits at 30 and 60 kg N ha^{-1} in 2010

Trait	Fertilization, kg N ha^{-1}	Price, € kg^{-1}	Value, € ha^{-1}	Plant height, cm	Number of leaves	Ninth leaf size, cm^2
Yield, kg ha^{-1}	30	0.59**	0.96**	0.39*	-0.23 n.s.	0.83**
	60	0.37*	0.96**	0.15 n.s.	-0.40*	0.79**
Price, € kg^{-1}	30		0.78**	0.18 n.s.	0.32 n.s.	0.42*
	60		0.62**	0.13 n.s.	0.15 n.s.	0.19 n.s.
Value, € ha^{-1}	30			0.35 n.s.	-0.06 n.s.	0.77**
	60			0.16 n.s.	-0.28 n.s.	0.72**
Plant height, cm	30				-0.34 n.s.	0.43*
	60				-0.24 n.s.	0.11 n.s.
Number of leaves	30					-0.29 n.s.
	60					-0.47**

* - significant at p<0.05; **- significant at p<0.01; n.s. - not significant

Table 6
Pearson's correlation coefficients among the studied traits at 30 and 60 kg N ha^{-1} in 2011

Trait	Fertilization, kg N ha^{-1}	Price, € kg^{-1}	Value, € ha^{-1}	Plant height, cm	Number of leaves	Ninth leaf size, cm^2
Yield, kg ha^{-1}	30	0.31 n.s.	0.90**	0.71**	0.52**	0.59**
	60	0.61**	0.95**	0.79**	0.53**	0.59**
Price, € kg^{-1}	30		0.69**	0.34 n.s.	0.43*	-0.22 n.s.
	60		0.83**	0.55**	0.44*	0.14 n.s.
Value, € ha^{-1}	30			0.68**	0.58**	0.34 n.s.
	60			0.77**	0.56**	0.46**
Plant height, cm	30				0.74**	0.47**
	60				0.63**	0.57**
Number of leaves	30					0.04 n.s.
	60					-0.02 n.s.

* - significant at p<0.05; **- significant at p<0.01; n.s. - not significant

and leaf number, as well as by the length of the vegetation period (Kozumplik and Šmalcelj 1980). Positive correlations between yield and leaf length, yield and leaf width, and yield and plant height were found earlier by Matzinger and Wernsman (1970) as well. Šarčević et al. (2013) studied the genetic improvement of Croatian flue-cured tobacco cultivars released from 1983 to 2007 and discussed that the improved yield of the newer cultivars might be explained by increases in leaf number, similarly like Bowman and Sisson (2000) and, possibly to improved leaf body which was in the present study not measured. Different patterns of correlations between the two experimental years suggest that in wet seasons higher influence on yield could be expected from the leaf size, while during normal seasons previously determined influence of leaf number and plant height on yield could be expected.

Conclusions

The examined cultivars reacted similarly to the applied N fertilization rates, 30 and 60 kg N ha^{-1}. The environmental factors, primarily precipitation, also had effect on the measured characteristics. Nitrogen N fertilization rate for flue-cured tobacco fertilization in Croatia should not be lower than currently recommended N rate, which is about 45 kg N ha^{-1}. Studied cultivars during two experimental years showed the potential for effective utilization of 60 kg N ha^{-1} without negative effect on the cured leaf price and quality. The 60 kg N ha^{-1} could also compensate the loss of nitrogen caused by leaching during the wet seasons. The results of the correlation analysis suggest that during wet season's leaf size could have high effect on yield of flue-cured tobacco, while during normal seasons the previously determined effect of leaf number and plant height on yield could be expected. In term of soil type, cultivars showed similar performance indicating that they could be equally grown on both heavier and sandy soils.

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