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Correlation and sequential path analysis of some agronomic traits in tobacco (*Nicotiana tabaccum* L.) to improve dry leaf yield

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Abstract

Tobacco (*Nicotiana tabaccum*) is an industrial plant because of its dry leaves which are used in cigarette and cigar making. Due to the economic significance of dry leaves, the correlated response of dry leaves with other traits is of vital importance in tobacco breeding programs. In the present research, sequential path analysis is used to determine the interrelationships among dry leaf yield and 7 related traits comprising fresh leaf yield, plant height, leaf number, leaf width, leaf length, stem girth and days to 50% flowering. One hundred tobacco genotypes were grown in a lattice design with 2 replications to determine the important components of dry leaf yield. Correlation coefficient analysis revealed that dry leaf yield was positively and significantly correlated with all the traits studied. Sequential path analysis localized the studied morphological traits in three orders according to their contribution to dry leaf yield. Among studied traits, only fresh leaf yield was identified as a first order trait whereas stem girth and leaf length presented maximum direct effects in second and third orders, respectively. According to bootstrap analysis, all direct effects had low standard errors showing the robustness of the sequential path model in tobacco. The complete results show that fresh leaf yield, leaf length, leaf number and stem girth could be used as selection criteria to improve dry leaf yield in tobacco.

Keywords: tobacco, sequential path analysis, bootstrap analyses, correlation, yield related traits.
Abbreviations: DF, Days to %50 Flowering; DLY, Dry Leaf Yield; FLY, Fresh Leaf Yield; LL, Leaf Length; LN, Leaf Number; LW, Leaf Width; PH, Plant Height; SG, Stem Girth.

Introduction

Tobacco (*Nicotiana tabaccum* L.) is an industrial plant and polyploid in nature. Numerous types of tobacco are defined to a large extent by region of production, intended use in manufacturing (e.g., cigar filler and cigar wrapper), method of curing (dark air-cured and fire-cured) as well as their morphological and biochemical characteristics (Ren and Timko, 2001). The most important aim of tobacco breeding programs is improving dry leaf yield of the plant which is a complex trait associated with many interrelated components. Dry leaf yield in tobacco is a quantitative trait largely influenced by the environment and hence has a low heritability (Xiao et al., 2007). Therefore, the response to direct selection for dry leaf yield may be unpredictable unless there is good control of environmental variation (Sabaghnia et al., 2010). Commonly, plant breeders prefer to select for yield related traits that indirectly increase yield. According to the literature, indirect selection by yield related traits such as plant height, leaf area index, number of leaves, leaf length and flowering date can increase tobacco dry leaf yield (Legg and Collins, 1975). White et al. (1979) used simple correlation analysis based on agronomic, physical and chemical characteristics to show the interrelationships among flue-cured tobacco genotypes and indicated that all agronomic traits present positive correlations with dry leaf yield. Honarnejad and Shoai-Deylami (2004) found high significant correlations between dry leaf yield and agronomic traits except for plant height and leaf number in a F2 population of tobacco. Wenping et al., (2009) reported that the most strongly correlated traits with dry leaf yield are leaf number and leaf length. Since increasing numbers of independent variables can compound apparent interdependence, therefore, correlations may be insufficient to explain the associations in a way to enable breeders to decide on a direct or indirect selection strategy. Path analysis is considered a part of structural equation modeling (SEM) usually used to identify and clarify relationships among different traits (Kang et al., 1983; Kozak and Azevedo, 2010). In the conventional approach, all predictor variables are organized as first-order and then their direct and indirect effects on the response variable are analyzed (Gopal et al. 1994; Mokhtassi Bidgoli et al., 2006). Samonte et al. (1998) adopted a sequential path analysis to determine the relationships between yield and its related traits in rice (*Oryza sativa* L.) by locating and analyzing various predictor variables in first, second and third-order paths. Interrelationships between yield and yield components using this model were identified in maize, potato, canola and wheat (Mohammadi et al., 2003; Asghari-Zakaria et al., 2009;

Table 1. Tobacco genotypes used in the experiment.

Code	Genotype	Code	Genotype	Code	Genotype
1	TR1	34	TK23	67	SPT403
2	TR21	35	PZ17	68	SPT405
3	TR93	36	Orumia 205	69	SPT406
4	ch.T 269-12	37	Orumia 209	70	SPT408
5	TS8	38	Orumia 379	71	SPT409
6	FK 40-1	39	Erzeogovina	72	SPT410
7	Jahrom 12	40	PBD 6 X Mut 4 (f1)	73	SPT412
8	Samson1	41	SPT 414XPobeda	74	SPT413
9	Samsoun 959	42	Trabzon	75	SPT420
10	Samson Katerini	43	Line 20	76	SPT430
11	Samsoun Dere	44	Jahrom 14	77	SPT432
12	TYK-Kula	45	ch.Trabzon 269-12B	78	SPT433
13	Alborz 23	46	KP14/a	79	SPT434
14	SS289-2	47	Nevrokop	80	SPT436
15	Basma 12-2	48	Mutant No2	81	SPT439
16	Basma16-10	49	Mutant No3	82	SPT441
17	Basma 104-1	50	Jahrom 15	83	PD324
18	Basma 181-8	51	L16	84	PD325
19	Basma Mahalades	52	ch.t 209-12e	85	PD328
20	KB	53	Xanthi	86	PD329
21	GD165	54	ch.T.283-8	87	PD336
22	D566	55	TK28	88	PD345
23	Pobeda 2	56	ch.T.266-6	89	PD364
24	Pobeda 3	57	Trabzon No 23	90	PD371
25	Krumovgrad 42	58	CH.T.273.38	91	PD371
26	Krumovgrad N.H.H.659	59	Pobeda 1	92	PD381
27	Harmanli 11	60	PL7	93	Mutant 4
28	Immuni 3000	61	L17	94	CHT269-12XFK401
29	Kharmanli 163	62	Melnik 261	95	TK FK40-1(Mutant GH)
30	Izmir	63	KB101	96	TB22
31	Kuklen 6	64	Trabzon H.T.1	97	Krumovgrad
32	Nevrokop 261	65	Trimph (Type Virginia)	98	Krumovgrad kanti
33	Ploudiv	66	Basma S.31	99	Ohdaruma
				100	Matianus

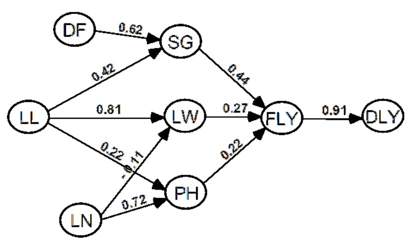

Fig 1. Sequential path analysis diagram depicting interrelationships between various traits contributing to dry leaf yield of tobacco. Where DF, Days to %50 Flowering; DLY, Dry Leaf Yield; FLY, Fresh Leaf Yield; LL, Leaf Length; LN, Leaf Number; LW, Leaf Width; PH, Plant Height; SG, Stem Girth.

Sabaghnia et al., 2010). Simple correlation values among 13 leaf characteristics and 32 smoke characteristics were determined and multiple regression equations were analyzed in selected variables and the most important characteristics were identified (Tso et al., 1982). Using conventional path analysis in tobacco, Honarnejad and Shoai-Deylami (2004) showed that traits including leaf area index and percent of dry matter are effective influences on dry leaf yield. To our knowledge, there is no report on the association of traits with sequential path analysis in tobacco. Therefore, the main objective of our research is to determine the associations between dry leaf yield and some related agronomic traits in tobacco genotypes by using correlation and sequential path model to identify traits that may be useful in breeding high-yielding lines.

Results

Analysis of variance revealed significant differences between tobacco genotypes for all the traits studied (data not presented). Simple correlation coefficients between traits were calculated. The overall phenotypic correlations among the traits are presented in Table 2. Dry leaf yield was highly and positively correlated with all of the measured traits. The highest value was observed between dry leaf yield and fresh leaf yield (0.96) and the lowest was between dry leaf yield and leaf number (0.29). Traits comprising fresh leaf yield, stem girth and days to 50% flowering, were significantly and positively correlated with all measured traits. As shown in Table 2, there was a statistically significant and positive correlation between plant height and other tobacco traits

Table 2. Pearson correlation coefficients between 8 traits of 100 oriental tobacco genotypes

Traits	LN	LL	LW	FLY	DLY	PH	SG	DF
LN	1							
LL	-0.09^{ns}	1						
LW	-0.19^{ns}	0.82**	1					
FLY	0.31**	0.57**	0.54**	1				
DLY	0.29**	0.59**	0.57**	0.96**	1			
PH	0.70**	0.15^{ns}	0.12^{ns}	0.46**	0.47**	1		
SG	0.40**	0.70**	0.54**	0.70**	0.66**	0.46**	1	
DF	0.66**	0.44**	0.32**	0.63**	0.63**	0.62**	0.81**	1

Where DF, Days to %50 Flowering; DLY, Dry Leaf Yield; FLY, Fresh Leaf Yield; LL, Leaf Length; LN, Leaf Number; LW, Leaf Width; PH, Plant Height; SG, Stem Girth.

except for leaf length and leaf width. Both leaf length and leaf width were also negatively but not significantly correlated with leaf number. Processing of the data by the sequential path coefficient analysis enabled the partitioning of the direct and indirect effects of individual dry leaf yield components and identification of dry leaf yield attributes as selection criteria. All studied traits could be organized based on their contribution to dry leaf yield (Fig. 1). Among studied traits, fresh leaf yield produced the highest direct effect (0.91) compared to other traits (Fig. 1 and Table 3). Therefore, fresh leaf yield could be appropriate as a first-order variable while its adjusted coefficient of determination (0.92) represents the influence of this trait on total variability of dry leaf yield (Table 3). The traits including stem girth, leaf width and plant height positively influenced the fresh leaf yield and were established as second-order variables (Fig. 1 and Table 3). Considering the path diagram (Fig. 1), leaf length, leaf number and days to 50% flowering were established as third-order variables. Thus, days to 50% flowering may be considered as the third order variable in relation to dry leaf yield in tobacco. Leaf length and leaf number positively (0.81) and negatively (-0.11) influenced leaf width respectively; meanwhile, leaf width had no direct association with days to 50% flowering (Fig. 1). Leaf number also had positive (0.72) and negative direct effects (-0.11) on plant height and leaf width, respectively (Fig. 1 and Table 3). In each case, for validation of each direct effect, 1000 bootstrap samples were done to estimate mean and standard errors of direct effects. Bootstrap values indicated that the estimated means of direct effects were closely in accordance with observed direct effects (Table 3). Among second order variables, there were some indirect effects for stem girth through leaf width (0.25) and plant height (0.15) on fresh leaf yield (Table 4). However, both leaf width and plant height showed indirect effects via stem girth on fresh leaf yield. These results show that stem girth is one of the most important agronomic traits to predict dry leaf yield in tobacco. Middling third-order variables, indirect effects of leaf length and leaf number through each other on plant height and leaf width, are narrow and negative (Table 4). Indirect effects of days to 50% flowering through leaf length (0.19) and indirect effects of leaf length through days to 50% flowering (0.27) on stem girth as responsible variables were positive (Table 4).

Discussion

Significant differences between tobacco genotypes for the traits studied here show the possibility of selection between genotypes for the purpose of improving tobacco. Zeba and Isbat (2011) described similar differences regarding the morphological traits in tobacco. From the plant breeder's viewpoint, genetic variation for yield and its components is important but correlation and path coefficient analysis are necessary for better understanding of the ways that dry leaf yield components may influence it. According to our results, fresh leaf yield is the best criterion for improving dry leaf yield in tobacco genotypes. On the other hand, late flowering is accompanied with increasing plant height and obviously this is followed by increasing the number of leaves and leaf width, respectively. Our results concerning simple correlations are in accordance with previous reports in other tobacco genotypes (Legg and Collins, 1975). Honarnejad and Shoai-Deylami (2004) also reported a significant positive correlation between leaf area index and tobacco yield. Days to 50% flowering and stem girth could be considered as important traits in tobacco breeding because they show positive and significant correlations with other traits in this experiment (Table 2). Significant correlations between days to 50% flowering, leaf width and leaf number with dry leaf yield was reported by White et al. (1979). Wenping et al. (2009) found also positive and significant correlations of dry leaf yield with leaf length and leaf number. Significant phenotypic contributions to total sugar content of tobacco leaf were identified for agronomic traits including plant height, stem girth, leaf number, leaf length and leaf width (Xiao et al., 2007). In tobacco, all factors favoring high yield were found to possess negative associations with the leaf total alkaloids (White et al., 1979); therefore, development of high performance tobacco genotypes via selection could lead to potentially healthier products with low alkaloid. It is inferable from our sequential path analysis results that fresh leaf yield can be considered as a responsible variable for dry leaf yield in tobacco breeding programs. According to previous studies, using conventional path coefficient analysis on 10 traits of 24 flue-cured tobacco pure lines, fresh leaf yield also produced a strong impact on dry leaf yield (Wenping et al. 2009). Using conventional path model, Honarnejad and Shoai-Deylami (2004) showed that percentage of dry matter and leaf area index presented maximum direct effects on tobacco dry leaf yield. Other traits in our experiment which were located in second and third-orders could influence dry leaf yield through fresh leaf yield, as indicated by bootstrap analysis. Among second and third order variables, stem girth and leaf length could effectively be used to improve dry leaf yield in tobacco. These results are consistent with the reports of Mohammadi et al. (2003) and Sabaghnia et al. (2010) on maize and canola. Among second and third order variables, stem girth and leaf length could effectively used to improve dry leaf yield in tobacco. The relative contributions of each component in our study as determined using sequential path procedure are similar to

Table 3. Path coefficient and estimation of standard error values using bootstrap analysis

Response variable	Predictor variable	Direct effect	Adjusted R^2	Bootstrap	
				Mean	SE
DLY	FLY	0.91	0.92	0.96	0.04
FLY	SG	0.44	0.56	0.44	0.12
	LW	0.27		0.27	0.08
	PH	0.22		0.23	0.08
SG	DF	0.62	0.81	0.62	0.06
	LL	0.42		0.43	0.05
LW	LN	-0.11	0.69	-0.12	0.07
	LL	0.81		0.81	0.06
PH	LN	0.72	0.54	0.72	0.06
	LL	0.22		0.22	0.07

Where DF, Days to %50 Flowering; DLY, Dry Leaf Yield; FLY, Fresh Leaf Yield; LL, Leaf Length; LN, Leaf Number; LW, Leaf Width; PH, Plant Height; SG, Stem Girth.

Table 4. Indirect effects for the predictor variables grouped into first, second and third order variables

FLY				SG		
	SG	LW	PH		DF	LL
SG	-	0.15	0.1	DF	-	0.19
LW	0.25	-	0.03	LL	0.27	-
PH	0.21	0.03	-			

LW			PH		
	LL	LN		LL	LN
LL	-	0.01	LL	-	-.07
LN	-0.07	-	LN	-0.02	-

Where DF, Days to %50 Flowering; DLY, Dry Leaf Yield; FLY, Fresh Leaf Yield; LL, Leaf Length; LN, Leaf Number; LW, Leaf Width; PH, Plant Height; SG, Stem Girth.

those found in other crops (rice: Samonte et al., 1998; maize: Mohammadi et al., 2003; potato: Asghari-Zakaria et al., 2007; Sabaghnia et al., 2010).

Materials and methods

Plant materials

One hundred tobacco genotypes were evaluated in Urmia Tobacco Research Centre (UTRC), Urmia, Iran (Table 1). The 'SPT' lines and 'Jahrom' lines known as 'Chopogh' and water pipe tobacco, respectively, are selected from local landraces. The 'PD' lines are recombinant inbred lines coming from the cross between Basma S. 31 and Dubec 566. Other genotypes used in this study are inbred lines from different countries introduced from CORESTA (Cooperation Center for Scientific Research Relative to Tobacco, Paris, France) collection or pure lines kindly provided by Iranian Tirtash Tobacco Research Centre (ITTRC).

Experimental design

Tobacco seeds were sown at a rate of approximately 5 g m-2 in beds. After sowing the seeds, a fine layer of well fermented and sieved sheep manure was spread on top of the beds. Tobacco seedlings were transplanted to plots in field when plants averaged about 12 cm in height. Plots of plants were established using a simple lattice design with two replications. Each plot comprised four lines 5 m long, with a spacing of 65 × 20 cm between lines and plants respectively. Traits including plant height (PH), stem girth (SG), leaf number (LN), leaf length (LL), leaf width (LW) and days to 50% flowering (DF) were measured on 5 random plants per plot. Dry leaf yield and fresh leaf yield per plant were evaluated using the mean all plants per plot exception of border effects.

Statistical analysis

Phenotypic correlations were calculated for all possible trait combinations, using the Pearson correlation coefficient. The sequential stepwise multiple regressions were performed to organize the predictor variables into first, second and the third order paths on the basis of their respective contributions to total variation in leaf dry weight using SPSS 14 (SPSS, 2005). Standard error of path coefficients was estimated by bootstrap analysis (Efron and Tibshirani, 1993) using S-Plus 2000 statistical package (MathSoft, 1999).

Conclusion

Simple correlations demonstrated very close relationships among dry leaf yield and other studied traits. Sequential path analysis identified fresh leaf yield as first order; stem girth, plant height and leaf width as second order; and days to %50 flowering, leaf length and leaf number as third order factors affecting dry leaf yield. Our results suggest that selection for dry leaf yield improvement should be based on fresh leaf yield, leaf length, leaf number and stem girth in tobacco.

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