

**SEQUENTIAL PATH ANALYSIS FOR DETERMINING INTERRELATIONSHIPS
BETWEEN YIELD AND RELATED TRAITS IN TOBACCO (*Nicotiana tabacum* L.)
UNDER NORMAL AND ABIOTIC STRESS CONDITIONS**

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In the present work the relationships between yield and its related traits were investigated in tobacco genotypes under normal and abiotic stress conditions (*Orobanchae aegyptiaca* weed) at Urmia Tobacco Research Centre, Iran, during 2006-2009 cropping seasons. The experimental design was a randomized complete block design (RCBD) with three replications in each condition every year. Analysis of variance revealed extent genetic variability among the genotypes for most of the traits studied. In comparison with normal condition, the mean value of studied traits decreased in stress condition. LAI and FD showed the maximum and minimum diminution in the mean values under stress condition compared to normal one so known as more sensitive and more tolerant traits, respectively. Based on CV values, the traits FD and DLYP showed the minimum and maximum variation among traits in both normal and stress conditions. Correlation analysis revealed significant and positive correlations between DLYP with all studied traits in both normal and stress conditions. Path analysis detected the traits including biomass, APDW and DWR as the first-order variables at normal condition and biomass, APDW, DWR and harvest index as the first-order variables under abiotic stress condition. Based on results, the traits such as biomass, APDW, DWR detected as more important factors in both conditions can be used in tobacco breeding programs for increasing yield.

Abbreviation: aerial part fresh weight without leaves weight (APFW), aerial part dry weight without leaves weight (APDW), biomass (BIO), coefficient of variation (CV), dry weight of root (DWR), flowering date (FD), fresh weight of leaf (FWL), fresh weight of

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root (FWR), harvest index (HI), leaf area index (LAI), dry leaf yield per plant (DLYP), number of leaf (NL), plant height (PH), randomized complete block design (RCBD), standard deviation (Std).

Key words: direct effect, indirect selection, multicollinearity, path analysis, predictor variables

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is the most important non-food crop in the world's agriculture and is cultivated in more than 100 countries on approximately 4.2 million hectares of crop land (KATARINA *et al.*, 2010). Beside leaves as economic part of plant, seeds contain 38% of non-edible oil which could be an appropriate substitute for diesel fuel (GIANNELOS *et al.*, 2002). Nevertheless, because of the high nicotine content, tobacco stalk can be used to produce pesticides (RADOJIČIĆ *et al.*, 2008). Numerous types of tobacco are defined by different criteria such as region of production, intended use in cigar (i.e., filler, binder and wrapper) and cigarette manufacturing, method of curing (flue-, air-, sun- and fire-cured tobacco) as well as morphological and biochemical characteristics (i.e., aromatic fire-cured, bright leaf tobacco, Burley tobacco, Turkish or oriental tobacco) (REN and TIMKO, 2001). Turkish or oriental tobacco is a sun-cured, highly aromatic, and small-leafed type which is grown in Iran, Turkey, Greece, Bulgaria, Lebanon and the Republic of Macedonia. It has the ability to grow in low fertility soils (DAVIS and NIELSEN, 1999). In order to get an American Blend type of cigarette, it is mixed with more robust tobacco such as Virginia and Burley.

Egyptian broomrape (*Orobanche aegyptiaca*) is one of the most important parasitic weeds in Iran (HASANNEJAD *et al.*, 2006), infesting crops in the Solanaceae and Cucurbiaceae families. It is also the most serious holoparasite on tobacco, with yield losses up to 100% having been recorded (ASHRAFI *et al.*, 2008). Several strategies such as cultural practices, chemical control have been developed to control broomrape but none was successful (MARIAM and SUWANKETNIKOM, 2004). For instance, applying herbicides is extremely difficult, because the holoparasite is directly connected to host plants, is predominantly subterranean, and produces numerous, durable, and small seeds (BUSCHMANN *et al.*, 2005). Given such a scenario, the development of Orobanche-resistant varieties is a more efficient way of limiting the effects of this parasitic genus on crops (RUBIALES *et al.*, 2003a; PÉREZ-DE-LUQUE *et al.*, 2008). Breeding for resistance is the most economic, feasible, and environmentally friendly method of control. Host genetic resistance is generally considered critical to successful integrated pest management programs (GOLDWASSER *et al.*, 2001; JOEL, 2000; MOROZOV *et al.*, 2000; RUBIALES *et al.*, 2003b). Resistance to broomrape is scarce and complex in nature (RUBIALES *et al.*, 2003a; MARIAM and SUWANKETNIKOM, 2004). Resistance to Orobanche is the result of the combination of several mechanisms including low induction of parasite seed germination, darkening the infection site on the host root that prevents parasite establishment and a reduced development of established parasite tubercles (RUBIALES *et al.*, 2003a). Considerable levels of resistance have been found in some accessions of host plants against parasites in some host-parasite systems such as sorghum-*Striga hermonthica*, cowpea-*S. gesnerioides* (RUBIALES *et al.*, 2003b) and chickpea-*Orobanche crenata* (RUBIALES *et al.*, 2003b).

Development of new tobacco cultivars requires efficient tools to monitor traits association in breeding programs. The efficiency of a breeding program depends mainly on the direction of the correlation between yield and its components and the relative importance of each component

contributing to yield. The interrelationships among plant yield and other traits may be varied in different growth conditions. BARADARAN FIROUZABADI *et al.* (2011) studied the interrelationships between white sugar yield and related traits in sugar beet under normal and drought stress conditions and reported that the root diameter, sugar yield, molasses content and sugar content traits have the most direct effect (0.005, 0.83, -0.09 and 0.19, respectively) on white sugar yield under normal condition while sugar content (0.09), sugar yield (1.1) and molasses content (-0.23) were the most effective traits on white sugar yield under drought stress condition. SABAGHNIA *et al.* (2010) reported that the main stem length (MSL) and 1,000-seed weight (TSW) traits with 0.65 and 0.13 direct effects, respectively, involved in the seed yield variation as the first-order variables under non-stress environment while the plant height (PH) and 1000-seed weight (TSW) traits with 0.37 and 0.65 direct effect, respectively accounted for nearly 56% of the seed yield variation as the first order variables under water-stress environment.

Different statistical techniques such as correlation, regression, path, factor and cluster analyses are used in modeling crops yield (LEILAH and AL-KHATEEB, 2005). Simple correlation studies might be informative in selection steps, but fails to produce a rather perfect picture about relative importance of each trait. To address this matter, multivariate statistical analyses such as path analysis may unravel the interrelationships among traits. Path coefficient analysis is widely used in plant breeding programs to determine the nature of the relationships between yield and yield components. The method helps breeders to select the best genotypes based on yield and related traits (AMJAD *et al.*, 2009). In the most of path analysis studies, researchers consider the predictor characters as first-order variables in order to analyze their effects over a dependent variable such as yield. This approach might be affected from multicollinearity of variables, and possibly make difficulties in interpretation of the actual contribution of variables (MOHAMMADI *et al.*, 2003; DALKANI *et al.*, 2011). DALKANI *et al.* (2011) adopted a sequential path analysis to determine the relationships between yield and its related traits in Ajowan (*Carum Copticum* L.) by locating and analyzing various predictor variables in first and second-order paths. Several reports on using sequential path coefficient analysis in plants such as maize (MOHAMMADI *et al.*, 2003), switch grass (DAS *et al.*, 2004), potato (ASGHARI-ZAKARIA *et al.*, 2007), melon (FEYZIAN *et al.*, 2009), Ajowan (DALKANI *et al.*, 2011), and tobacco (HATAMI MALEKI *et al.*, 2011) are available.

In the present work, our aims are to determine the relationships between yield and its related traits in tobacco at normal and abiotic stress condition via both simple correlation and sequential path analysis and further to demonstrate the advantage of this method over conventional path analysis and introduce appropriate indicators to improve tobacco yield in the breeding programs.

MATERIALS AND METHODS

Ninety one oriental and semi oriental tobacco genotypes (Table 1) were studied separately under normal and abiotic stress conditions at a greenhouse in Urmia Tobacco Research Centre, Iran, twice at 2006-2007 and 2007-2008. The 'SPT' lines known as 'Chopogh' were selected from local landraces in Urmia Tobacco Research Centre. The 'PD' and 'OR' lines are recombinant inbred lines (RILs) 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 the CORESTA (Cooperation Center for Scientific Research Relative to Tobacco, Paris, France) collection or pure lines kindly provided by Iranian Tirtash Tobacco Research Centre (TTRC).

Seeds of genotypes were sown at a rate of approximately 5 g m⁻² in bed. After sowing the seeds, a fine layer of well fermented and sieved sheep manure was spread on top of beds. Tobacco

seedlings on 12 cm in height were transplanted to ceramic plant pots which filled with 10 kg of sterilized soil. We used soil that was sampled from alfalfa farm and was representative of locations in northwest Iran where the oriental tobacco type is planted. In abiotic stress conditions the soil of each pot was artificially mixed with 0.06 g Egyptian broomrape (*Orobanche aegyptiaca*) seeds. The experiments were arranged in randomized complete block design (RCBD) with three replications. Each replication consisted of one pot with one plant. Harvests were done at technical maturity. The leaves were sun-cured. The plants were not topped as is common with most other tobacco types (such as: Virginia and Burley). Different traits such as: flowering date (FD), plant height (PH), number of leaf (NL), leaf area index (LAI), fresh weight of leaf (FWL), fresh weight of root (FWR), dry weight of root (DWR), aerial part fresh weight without leaves weight (APFW), aerial part dry weight without leaves weight (APDW), biomass (BIO), harvest index (HI), dry leaf yield per plant (DLYP) were measured.

Table 1. Name of tobacco genotypes used in the present experiment

Code Genotype	Code Genotype	Code Genotype	Code Genotype	Code Genotype	Code Genotype
1 Kharmanli163	20 Pz 17	39 Kramograd N.H.H. 565	58 P.D.345	77 SPT 439	
2 Neverlop	21 K.P.Ha	40 OR-379	59 P.D.364	78 SPT 441	
3 Trabozan	22 K.B	41 ² C.H.T.209.12e	60 P.D.365	79 ⁵ Isfahani2	
4 Kromograid	23 G.D.165	42 C.H.T.209.12e×F.K.40-1	61 P.D.371	80 SPT 413	
5 Basma S.31	24 H.T.I	43 C.H.T.266-6	62 P.D.381	81 ⁵ Isfahani	
6 Triumph	25 OR-345	44 C.H.T.283-8	63 ⁴ SPT 403	82 ⁵ Jahrom14	
7 Xanthi	26 T.K.23	45 C.H.T.273-38	64 SPT 405	83 ⁵ Borazjan	
8 Matianus	27 L 16	46 Basma 12-2	65 SPT 406	84 L17	
9 Immni 3000	28 Izmir 7	47 Basma 16-10	66 SPT 408	85 ⁵ Balouch	
10 Melkin 261	29 ¹ Mutant 3	48 Basma 104-1	67 SPT 409	86 ⁵ Lengeh	
11 Try-Kula	30 ¹ Mutant 4	49 Basma 181-8	68 SPT 410	87 ⁵ Saderati	
12 Ss-289-2	31 Pobeda 1	50 C.H.T.269-12e	69 SPT 412	88 ⁵ Araghi	
13 Ohdaruma	32 Pobeda 2	51 Samsun katerini	70 ⁵ Isfahani	89 ⁵ Shahrodi	
14 Ploudive 58	33 Rustica	52 ³ P.D.324	71 SPT 420	90 T.K.L	
15 Line 20	34 Samsun 1	53 P.D.325	72 SPT 430	91 Zichna	
16 T-B-22	35 Samsun 959	54 P.D.406	73 SPT 432		
17 Ts 8	36 Samsun Dere	55 P.D.328	74 SPT 433		
18 Alborz 23	37 Izmir	56 P.D.329	75 SPT 434		
19 F.K.40-1	38 OR-205	57 P.D.336	76 SPT 436		

¹Early-maturing mutants developed in Urmia Tobacco Research Center. ²The "C.H.T." lines known as Tikolak are semi oriental tobacco developed in Tirtash Tobacco Research Centre (Iran). ³The "PD" and "OR" lines are recombinant inbred lines (RILs) derived from the cross between Basma S. 31 and Dubec 566 in Urmia Tobacco Research Centre. ⁴The "SPT" lines known as "Chopogh" tobacco are inbred lines selected from local landraces by single-plant or pure-line selection

method in Urmia Tobacco Research Centre. ⁵Iranian water pipe's tobacco line selected from local landraces by single-plant or pure-line selection method in Urmia Tobacco Research Centre.

Statistical analyses

Normality test of data was performed with SPSS version 21 software. It was tried to use the sterile soil (soil naturally without broomrape seeds) in all experiments, but broomrape was observed in some pots under normal conditions. In this case, it was tried to disappear the broomrape effects by covariance analysis and make the quite similar condition for all pots. In analysis of covariance, three traits (broomrape number, dry and fresh weight of broomrape) were noted as covariate in the model. Statistical analyses were continued with corrected means of genotypes in the normal conditions. Simple correlations between traits were calculated at each one of growth conditions by using the genotypes means on two years. Stepwise regression was applied to determine the interrelationships between independent and dependent variables and further to reveal the first-, and second-paths of predictor variables. The independent variables were grouped according to their contribution to yield considering the minimal multicollinearity effect. The first order independent variables were the traits with highest regression coefficients in the stepwise regression. Consecutively, these independent traits were considered as dependent variables to the remaining traits and second stepwise regressions were performed to reveal the second order independent traits. The multicollinearity effect of variables was measured by variance inflation factor (VIF) and tolerance value (TV) in the SPSS software (HATAMI MALEKI *et al.*, 2011). $TV=1-R_i^2$, where R_i^2 is the coefficient of determination in the prediction of variable i by the other predictor variables. $VIF=1/(1-R_i^2)$ representing the extent of the effects of other independent variables on the variance of the selected independent variable. The high degree of multicollinearity can be explained by values greater than 10 for VIF and values smaller than 0.1 for TV (WANG *et al.*, 1999).

RESULTS AND DISCUSSION

Dry leaf yield per plant (DLYP) and flowering date (FD) presented the highest and lowest phenotypic coefficient of variation (%CV) among the studied traits in both growth conditions. Genetic variability is the raw material of crop breeding industry on which selection acts to evolve superior genotypes. A higher variation for a character in the breeding materials increases the ability for selection toward its improvement. Based on simple statistic parameters, the average of all studied traits except for harvest index (HI) were less under abiotic condition than normal state (Table 2). Among the studied traits, aerial part fresh weight without leaves weight (APFW) with $d=34.77\%$ and flowering date (FD) with $d=0.2\%$ presented respectively maximum and minimum diminution in the trait value under stress condition compared to normal state. So these traits can be considered as more sensitive and tolerant traits to Egyptian broomrape parasite. LIQIN *et al.* (2012) reported that abiotic stress is an important factor which affects plant growth and yield.

Correlation coefficient analysis

Significant and positive correlation were observed between dry leaf yield per plant (DLYP) and other traits; among these biomass (BIO) with $r = 0.92^{**}$ and 0.90^{**} and fresh weight of leaf (FWL) with $r = 0.90^{**}$ and 0.85^{**} under normal and abiotic stress conditions, respectively, showed maximum correlation with dry leaf yield per plant (DLYP); and flowering date (FD) with $r = 0.32^{**}$ and 0.34^{**} and plant height (PH) with $r = 0.34^{**}$ and 0.31^{**} under normal and abiotic stress conditions, respectively, showed minimum correlation with dry leaf yield per plant (DLYP) (Table

3). Correlation coefficient is a measure of interdependence between a pair of characters (ALI *et al.*, 2009).

Table 2. Estimate of statistical parameters for studied traits in tobacco under normal and abiotic stress (*Orobanche aegyptiaca* weed) conditions

Traits	Minimum		Maximum		Rang		Average		D ^a	Std		CV%	
	Normal Stress		Normal Stress	Normal Stress									
FD	40.83	42.3	80.84	78.3	40.00	36	60.92	60.8	0.20	8.23	6.7	13.51	11
PH	23.06	27	113.20	95.7	90.14	68.7	63.94	53.5	16.33	18.88	16	29.52	30
NL	9.21	9	40.77	33	31.56	24	22.67	20.2	10.91	6.94	6.4	30.59	31.6
LAI	157.06	229	4699.56	3343.6	4542.50	3114.6	1802.79	1392.4	22.76	919.61	650.3	51.01	46.7
FWL	10.94	11.2	182.05	105.8	171.11	94.5	58.21	44.9	22.87	35.43	21.8	60.86	48.5
FWR	9.24	2.07	60.30	40	51.06	38	29.98	22.8	23.95	11.98	8.7	39.95	38.2
DWR	2.26	1.37	22.15	12.9	19.89	11.5	9.84	6.9	29.88	4.67	2.7	47.41	38.9
APFW	15.98	11.6	177.90	118.6	161.93	107	71.75	46.8	34.77	34.45	22.2	48.02	47.5
APDW	4.36	3.6	43.79	32.5	39.43	29	17.59	11.6	34.04	7.92	5.6	45.05	48.1
BIO	11.58	10	100.81	72.2	89.23	62.2	41.81	29.7	28.96	19.55	13	46.76	43.9
HI	9.40	17.7	56.27	58.8	46.88	41.1	32.14	36.7	-14.18	8.59	7.4	26.72	20.2
DLYP	2.52	3.3	47.69	34.9	45.18	31.6	14.38	11	23.52	9.60	6.4	66.77	58.4

FD: flowering date, PH: plant height, NL: number of leaf, LAI: leaf area index, FWL: fresh weight of leaf, FWR: fresh weight of root, DWR: dry weight of root, APFW: aerial part fresh weight without leaves weight, APDW: aerial part dry weight without leaves weight, BIO: biomass, HI: harvest index, DLYP: dry leaf yield per plant. CV: coefficient of variation, Std: standard deviation.

$$d = \frac{\bar{X}_{Normal} - \bar{X}_{Biotic}}{\bar{X}_{Normal}} \times 100.$$

Table 3. Correlation coefficient among studied traits in tobacco genotypes under normal (above diagonal) and abiotic stress (*Orobanche aegyptiaca* weed) (below diagonal) conditions.

Traits	FD	PH	NL	LAI	FWL	FWR	DWR	APFW	APDW	BIO	HI	DLYP
FD	1	0.62**	0.48**	0.33**	0.34**	0.37**	0.37**	0.31**	0.30**	0.37**	0.16	0.32**
PH	0.58**	1	0.73**	0.40**	0.41**	0.60**	0.56**	0.60**	0.57**	0.53**	-0.18	0.34**
NL	0.60**	0.81**	1	0.60**	0.59**	0.65**	0.62**	0.72**	0.72**	0.72**	0.17	0.57**
LAI	0.40**	0.47**	0.70**	1	0.72**	0.60**	0.51**	0.70**	0.67**	0.73**	0.39**	0.68**
FWL	0.46**	0.43**	0.70**	0.80**	1	0.71**	0.62**	0.76**	0.71**	0.88**	0.62**	0.90**
FWR	0.26*	0.47**	0.60**	0.65**	0.63**	1	0.85**	0.74**	0.63**	0.77**	0.28**	0.65**
DWR	0.36**	0.49**	0.58**	0.69**	0.64**	0.83**	1	0.65**	0.58**	0.80**	0.25*	0.66**
APFW	0.39**	0.62**	0.78**	0.75**	0.78**	0.68**	0.69**	1	0.88**	0.84**	0.18	0.66**
APDW	0.39**	0.55**	0.73**	0.65**	0.74**	0.61**	0.62**	0.90**	1	0.88**	0.13	0.68**
BIO	0.42**	0.49**	0.72**	0.75**	0.88**	0.71**	0.77**	0.87**	0.88**	1	0.47**	0.92**
HI	0.07	-0.11	0.09	0.24*	0.41**	0.01	-0.05	0.02	-0.02	0.28**	1	0.72**
DLYP	0.34**	0.31**	0.57**	0.66**	0.85**	0.54**	0.57**	0.67**	0.64**	0.90**	0.63**	1

FD: flowering date, PH: plant height, NL: number of leaf, LAI: leaf area index, FWL: fresh weight of leaf, FWR: fresh weight of root, DWR: dry weight of root, APFW: aerial part fresh weight without leaves weight, APDW: aerial part dry weight without leaves weight, BIO: biomass, HI: harvest index, DLYP: dry leaf yield per plant. * and **: significant at 0.05 and 0.01 probability level, respectively.

Knowledge on correlation is required to obtain the expected response of other characters when selection is applied to the character of interest in breeding programs (MOHAMMADI *et al.*, 2003). The positive association among various traits gave ability to tobacco breeders to select these traits simultaneously, especially under stress and normal conditions. The similar relations pattern was observed between dry leaf yield per plant (DLYP) and other studied traits in both normal and abiotic stress conditions. SABAGHNIYA *et al.* (2010) reported similar correlation pattern between yield and all studied traits in canola under normal and salt stress conditions.

Path analysis at normal condition

The path coefficient analysis has been utilized efficiently to clarify interrelationships between the dependent and several independent variables in plants (IQBAL *et al.*, 2009). The direct effects of the studied traits on dry leaf yield per plant (DLYP), the tolerance value (TV) and variance inflation factor (VIF) resulting from conventional path analysis are shown in Table 4. As high and significant correlations has been observed between biomass and fresh weight of leaf (FWL) ($r=0.85$), dry weight of root (DWR) ($r=0.83$), aerial part fresh weight without leaves weight (APFW) ($r=0.84$), aerial part dry weight without leaves weight (APDW) ($r=0.88$), as well as between APFW and APDW ($r=0.87$) and between fresh weight of root (FWR) and dry weight of root (DWR) ($r=0.86$) as predictor variables in conventional path model, it seems the results is affected with high multicollinearity. High multicollinearity was observed for biomass (VIF=58.85), DWAP (VIF=26.81) and DWR (VIF=12.51). MOHAMMADI *et al.*, 2003, DALKANI *et al.* (2011) and FEYZIAN *et al.* (2009) reported that the result coming from the conventional path model may be influenced by multicollinearity of predictor variables, particularly when the correlations among predictor variables are high. The presence of high multicollinearity for traits leads to unreal selection accompanied by frequent errors. For lessening the multicollinearity effects and complicating interrelationship between traits, sequential path analysis was applied. The direct effects of studied traits on dry leaf yield per plant (DLYP), tolerance and VIF values based on sequential path model are shown in Table 4. According to results, the VIF value for biomass, aerial part dry weight without leaves weight (APDW) and dry weight of root (DWR) decreased to 9.85, 5.77 and 3.88, respectively, so it is possible to have a clear and better understanding about interrelationship between traits in tobacco. BHAGOWATI and SAIKIA (2003) and TUNCTURK and ÇİFTÇİ (2005) stated that the most effective variables on dependent trait are being considered as the first-order predictors of response variable. MARJANOVIC-JEROMELA *et al.* (2007) and DALKANI *et al.* (2011) stated that the traits with high direct effect on yield and inserted to path model as the first-order variable can be selected as the main and effective traits to improve plant yield in breeding programs. Figure 1 present the sequential path analysis among the various variables and their relative contribution to dry leaf yield per plant (DLYP) under normal condition. As shown in Figure 1, biomass, dry weight of root (DWR) and aerial part dry weight without leaves weight (APDW) were inserted to model at the first stage and accounted for 100% of total variation of DLYP. Among these traits, biomass showed high direct and positive effect (2.04) on DLYP while APDW (-0.83) and DWR (-0.49) traits showed high direct and negative effects on DLYP. KOZAK

and KANG (2006) stated that the main advantage of path analysis is not only to identify the most important factor directly affecting a trait, but also to indicate how factors affect the trait indirectly through other factors. SABAGHNI *et al.* (2010) reported that the path analysis is able to compensate the severity negative and positive effects of traits on yield and identify actual yield components, subsequently.

Table 4. Variance inflation factor (VIF), tolerance values and direct effects for the predictor variables in conventional path (all predictor variables as first-order variables) and sequential path (predictors grouped according to first- and second- order variables) models in studied tobacco genotypes at normal condition.

Sequential path analysis							Conventional path analysis					
Dependent variable	Independent variable	R ²	Direct effect	Total indirect effect	Correlation coefficient	Tolerance value ^a	VIF ^b	Dependent variable	Independent variable	Direct effect	Tolerance value	VIF
DLYP ^a	BIO	1.00	2.04	-1.12	0.92**	0.10	9.59	DLYP	BIO	2.04	0.02	63.57
	APDW		-0.83	1.51	0.68**	0.19	5.30	APDW	-0.83	0.03	29.67	
	DWR		-0.49	1.15	0.66**	0.30	3.29	DWR	-0.49	0.08	13.16	
BIO	FWL	0.85	0.55	0.33	0.88**	0.42	2.35	FWL	0.00	0.12	8.55	
	APFW		0.28	0.56	0.84**	0.31	3.20	APFW	0.00	0.14	7.04	
	NL		0.19	0.53	0.72**	0.48	2.10	NL	0.00	0.29	3.44	
APDW	APFW	0.78	0.74	0.14	0.88**	0.48	2.08	FWR	0.00	0.18	5.44	
	NL		0.19	0.53	0.72**	0.48	2.08					
DWR	FWR	0.71	0.85	0.00	0.85**	1	1					

FD: flowering date, PH: plant height, NL: number of leaf, LAI: leaf area index, FWL: fresh weight of leaf, FWR: fresh weight of root, DWR: dry weight of root, APFW: aerial part fresh weight without leaves weight, APDW: aerial part dry weight without leaves weight, BIO: biomass, HI: harvest index, DLYP: dry leaf yield per plant. ^{a, b} A high degree of multicollinearity can be explained by values greater than 10 for VIF (variance inflation factor) and values smaller than 0.1 for tolerance value. * and **: significant at 0.05 and 0.01 probability level, respectively.

The important point is the high inconsistency between results from the correlation coefficient and sequential path analysis. As mentioned former, there were positive and significant correlation between dry leaf yield per plant (DLYP) and other studied traits. For instance the correlation between dry leaf yield per plant (DLYP) and aerial part dry weight without leaves weight (APDW) as well as between dry leaf yield per plant (DLYP) and dry weight of root (DWR) were 0.67 and 0.69, respectively. While the results from sequential path analysis indicated that both aerial part dry weight without leaves weight (APDW) and dry weight of root (DWR) traits had high direct and negative effects on dry leaf yield per plant (DLYP). However, APDW and DWR had high positive and indirect effect via biomass (1.51 and 1.2, respectively) on dry leaf yield per plant (DLYP) (Table 4). Biomass with high indirect effects via APDW and DWR could moderate the direct and negative effect of these traits on DLYP. The results are concordant with

DALKANI *et al.* (2011) studies. SABAGHNIA *et al.* (2010) also reported inconsistency between results from correlation coefficient and results from sequential path analysis. According to results it can be stated that sequential path analyses is a useful method to understand interrelations between traits and to determine the most effective traits on yield. Therefore, it is necessary to mention that the results from correlation coefficient analysis are mainly seductive and it needs to be followed by path analysis. MOHAMMADI *et al.* (2003) reported that the simple correlation analysis that relates yield to a single variable may not provide a complete understanding about the importance of each component in yield.

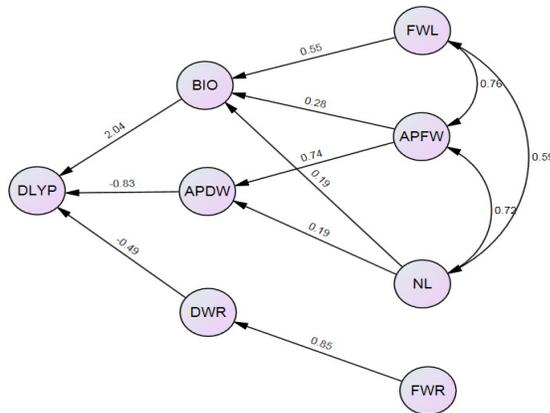


Figure 1. Sequential path model indicating interrelationships between dry leaf yield per plant and related characteristics at normal condition.

NL: number of leaf, FWL: fresh weight of leaf, FWR: fresh weight of root, DWR: dry weight of root, APFW: aerial part fresh weight without leaves weight, APDW: aerial part dry weight without leaves weight, BIO: biomass, DLYP: dry leaf yield per plant.

In the next step, biomass, dry weight of root (DWR) and aerial part dry weight without leaves weight (APDW) were considered as dependent variables and other traits as independent variables in order to construct the second order-variables. The fresh weight of leaf (FWL), aerial part fresh weight without leaves weight (APFW) and number of leaf (NL) traits accounted for 85% of total variation of biomass. Regarding to correlation coefficient as well as high positive direct and indirect effects of these traits to each other, these traits can improve biomass and subsequently dry leaf yield per plant (DLYP). APFW and NL accounted for 78% of total variation of APDW. Fresh weight of root (FWR) accounted for 71% of total variation of DWR solely. APFW and FWR with high direct effects (0.74 and 0.85, respectively) lead to increase APDW and DWR.

APFW, FWR, FWL and NL did not show any direct effect on dry leaf yield per plant (DLYP) in conventional path analysis, whereas in sequential path analysis, these traits had high positive indirect and direct effects on first order-variables and subsequently on dry leaf yield per plant (DLYP). Advantages of sequential path analysis to conventional path model is better

understanding the interrelationships among traits that have been shown in several researches works (MOHAMMADI *et al.*, 2003; AMJAD *et al.*, 2009; BAHRAMINEJAD *et al.*, 2011, HATAMI MALEKI *et al.*, 2011).

Path analysis at abiotic stress condition

Direct effects of traits on dry leaf yield per plant (DLYP), tolerance and VIF values based on conventional path analysis under abiotic stress condition are shown in Table 5. High multicollinearity were detected for some traits, especially for biomass (VIF=24.6) and aerial part dry weight without leaves weight (APDW) (VIF=12.4). Similar to normal condition, the results are therefore not valuable and multicollinearity must be reduced by appropriate model such as sequential path analysis. By applying sequential path model, the multicollinearity for traits decreased in comparison to conventional path model. For instance, VIF for biomass and APDW reduced to 20.4 and 2.98, respectively (Table 5). The diagram of sequential path analysis under abiotic stress condition is shown in Figure 2. As shown in diagram of sequential path model, biomass, harvest index, aerial part dry weight without leaves weight (APDW) and dry weight of root (DWR) were the first traits inserted in the model accounting for 98% of total variation of dry leaf yield per plant (DLYP) as dependent variable. Among these traits, biomass and harvest index had high direct and positive effect (1.44 and 0.2, respectively) on dry leaf yield per plant (DLYP). Two another traits (APDW and DWR) had high direct and negative effect (-0.24 and -0.47) on DLYP.

Table 5. Variance inflation factor (VIF), tolerance values and direct effects for the predictor variables in conventional path (all predictor variables as first-order variables) and sequential path (predictors grouped according to first- and second- order variables) models in studied tobacco genotypes at abiotic stress (*Orobanche aegyptiaca* weed) conditions

Sequential path analysis					Conventional path analysis							
Dependent variable	Independent variable	R ²	Direct effect	Total indirect effect	Correlation coefficient	Tolerance value ^a	VIF ^b	Dependent variable	Independent variable	Direct effect	Tolerance value	VIF
DLYP	BIO	0.98	1.44	-0.54	0.90**	0.05	20.4	DLYP	BIO	1.43	0.04	24.57
	HI	0.2	0.43	0.63**	0.34	2.98	HI		0.19	0.27	3.76	
	APDW	-0.47	1.11	0.64**	0.10	2.98	APDW		-0.47	0.08	12.4	
	DWR	-0.24	0.81	0.57**	0.21	4.75	DWR		-0.22	0.12	8.05	
BIO	FWL	0.86	0.47	0.41	0.88**	0.37	2.69	FWL	0.06	0.14	7.06	
	APFW	0.41	0.46	0.87**	0.34	2.98	APFW	-0.04	0.11	9.08		
	FWR	0.14	0.57	0.71**	0.51	1.96	FWR	-0.02	0.26	3.78		
HI	FWL	0.39	1.01	-0.6	0.41**	0.39	2.55	LAI	-0.01	0.24	4.15	
	APFW	-0.76	0.78	0.02	0.39	2.55						
APDW	APFW	0.81	0.9	0.0	0.90**	1	1					
DWR	FWR	0.73	0.66	0.17	0.83**	0.58	1.74					

FD: flowering date, PH: plant height, NL: number of leaf, LAI: leaf area index, FWL: fresh weight of leaf, FWR: fresh weight of root, DWR: dry weight of root, APFW: aerial part fresh weight without leaves weight, APDW: aerial part dry weight without leaves weight, BIO: biomass, HI: harvest index, DLYP: dry leaf yield per plant. ^{a, b} A high degree of multicollinearity can be explained by values greater than 10 for VIF (variance inflation factor) and values smaller than 0.1 for tolerance value. * and **: significant at 0.05 and 0.01 probability level, respectively.

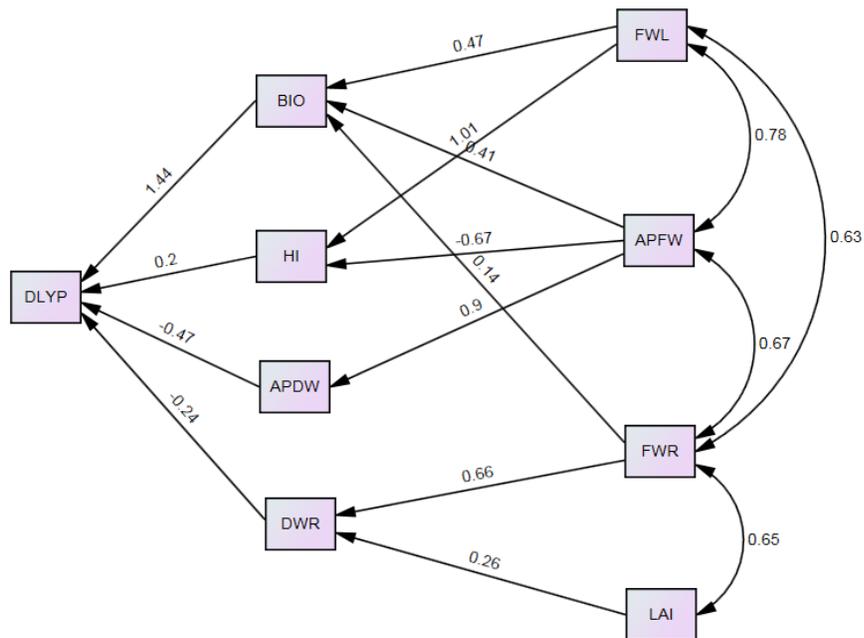


Figure 2. Sequential path model indicating interrelationships between dry leaf yield per plant and related characteristics at abiotic stress (*Orobanche aegyptiaca* weed) conditions.

LAI: leaf area index, FWL: fresh weight of leaf, FWR: fresh weight of root, DWR: dry weight of root, APFW: aerial part fresh weight without leaves weight, APDW: aerial part dry weight without leaves weight, BIO: biomass, HI: harvest index, DLYP: dry leaf yield per plant.

As mentioned former, dry leaf yield per plant (DLYP) had positive and significant correlation with the studied traits. Correlation between dry leaf yield per plant (DLYP) and aerial part dry weight without leaves weight (APDW) as well as between DLYP and DWR were 0.64 and 0.57, respectively. While the results from sequential path analysis indicated that aerial part dry weight without leaves weight (APDW) and dry weight of root (DWR) had high direct and negative effects on dry leaf yield per plant (DLYP). However, APDW and DWR had high positive and indirect effect via biomass and harvest index (1.51 and 1.2, respectively) on DLYP (Table 5). In other word, negative effect of aerial part dry weight without leaves weight (APDW) and dry weight of root (DWR) is compensated by biomass and harvest index traits. The study on the correlations among characters is not conclusive about cause of relationships (GONÇALVES *et al.*, 2003). Therefore, when there are many explanatory variables and a basic or dependent variable, it is necessary to use analyses such as path analysis that consider the correlations among the explanatory variables (DE ARAÚJO, *et al.*, 2012).

In the next step, biomass, harvest index, aerial part dry weight without leaves weight (APDW) and dry weight of root (DWR) were considered as dependent variables and other traits as independent. The results of second-order sequential path analysis (Table 5) showed that fresh weight of leaf (FWL), aerial part fresh weight without leaves weight (APFW) and fresh weight of root (FWR) accounting for 86% of total variation of biomass had high positive direct effect on biomass and consequently on dry leaf yield per plant (DLYP) under abiotic stress condition. YASIN and SINGH (2010) stated that plant breeders commonly prefer yield components that indirectly increase yield. Fresh weight of leaf (FWL) and aerial part fresh weight without leaves weight (APFW) accounted for 39% of total variation of harvest index. FWL had high positive and direct effect (1.01) on harvest index whereas APFW had high negative and direct (-0.76) effect on it. APFW accounted for 81% of total variation of aerial part dry weight without leaves weight (APDW). FWR and LAI accounted for 73% of total variation of dry weight of root (DWR).

The inconsistency of results from conventional and sequential path model has two reasons: firstly, due to multicollinearity effects in conventional path analysis as all direct effects are less in conventional path analysis compared to sequential path model. For instance, direct effect of harvest index and fresh weight of leaf (FWL) were 0.19 and 0.06 in conventional path analysis while they are 0.2 and 0.47 in sequential path analysis. Secondly, some traits had the negative effects on dry leaf yield per plant (DLYP) in conventional path analysis while these traits indicated positive effect on DLYP in sequential path model. For instant, fresh weight of root (FWR) had negative direct effect (-0.02) on DLYP in conventional path analysis while it showed positive direct effects on biomass and dry weight of root (DWR) (0.14 and 0.66, respectively) in sequential path analysis.

CONCLUSION

High multicollinearity was detected for some traits in conventional path analysis. Only focusing on correlation coefficient and conventional path analysis to interpret interrelating between traits is seducer. Sequential path analysis had important role in understanding interrelations between traits and determining the most effectiveness traits on tobacco yield. The important point of this study was differences in interrelationships among traits in both normal and abiotic stress conditions. Results of path analysis in normal condition showed that the first-order variables are biomass, aerial part dry weight without leaves weight (APDW) and dry weight of root (DWR) with high direct effects (2.04, -0.83 and -0.49 respectively) on dry leaf yield per plant (DLYP). Harvest index, fresh weight of root (FWR), aerial part fresh weight without leaves weight (APFW), fresh weight of leaf (FWL) and number of leaf (NL) were found to fit as second-order variables in this condition. In abiotic stress condition the first-order variables were biomass, aerial part dry weight without leaves weight (APDW), dry weight of root (DWR) and harvest index with 1.44, -0.47, -0.24 and 0.20 direct effects, respectively on dry leaf yield per plant (DLYP). Fresh weight of leaf (FWL), aerial part fresh weight without leaves weight (APFW), fresh weight of root (FWR) and leaf area index (LAI) were found to fit as second-order variables. Generally, biomass, harvest index, aerial part dry weight without leaves weight (APDW) and number of leaf (NL) were identified as important traits to improve tobacco yield in both normal and abiotic stress conditions. These traits can be used effectively as selection criteria for improving yield in tobacco breeding programs under both growth conditions.

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REFERENCES

- ALI, M.A., N.N. NAWAB, G. RASOOL, M. SALEEM (2009): Estimates of variability and correlation for some quantitative traits in *Cicer arietinum*. *J Agric Soc Sci.* 4:177-179.
- AMJAD, A.M., N.N. NOBEL, A. AMJAD, M. ZULKIFFAL, M. SAJJAD (2009): Evaluation of selection criteria in *Cicer arietinum* L. using correlation coefficients and path analysis. *Aust. J. Crop Sci.* 3: 65-70.
- ASGHARI-ZAKARIA, R., M. FATHI, D. HASAN-PANAH (2007): Sequential path analysis of yield components in potato. *J. Potato Res.* 49: 273-279.
- ASHRAFI, Z.Y., H.M. ALIZADEH, S. SADEGHI (2008): Effect of soil solarization on the control of Egyptian broomrape (*Orobanche aegyptiaca*) and yield improvement of cucumber (*Cucumis sativus*) grown in greenhouse. *Am. Eurasian J. Agric. Environ. Sci.* 4: 775-782.
- BAHRAMINEJAD, A., G. MOHAMMADI-NEJAD, M.A. KHADIR (2011): Genetic diversity evaluation of Cumin (*Cuminum cyminum* L.) based on phenotypic characteristics. *Aust. J. Crop Sci.* 5: 304-310.
- BARADARAN FIROUZABADI, M., N. FARROKHI, M. PARSAEYAN (2011): Sequential path analysis of some yield and quality components in sugar beet grown in normal and drought conditions. *Ital. J. Agron.* 6: 45-51
- BHAGOWATI, R.R., M. SAIKIA (2003): Character association and path coefficient analysis for yield attributes in open pollinated and hybrid true potato seed populations. *Crop Res.* 26: 286-290.
- BUSCHMANN, H., G. GONSIOR, J. SAUERBORN (2005): Pathogenicity of branched broomrape (*Orobanche ramosa*) populations on tobacco cultivars. *Plant Pathol.* 54: 650-656.
- DALKANI, M., R. DARVISHZADEH, A. HASSANI (2011): Correlation and sequential path analysis in Ajowan (*Carum copticum* L.). *J. Med. Plants Res.* 5: 211-216.
- DAS, K.M., R.G. FUENTES, C.M. TALIAFERRO (2004): Genetic variability and trait relationships in switch grass. *Crop Sci.* 44: 443-448.
- DAVIS, D.L., M.T. NIELSEN (1999): Tobacco: Production, chemistry and technology. Oxford, UK: CORESTA, Blackwell Science, 480 p.
- DE ARAÚJO, L.C., G. DE AMARAL GRAVINA, C. DORNELLES MARINHO, S.N. COSTA DE ALMEIDA, R. FIGUEIREDO DAHER, A.T. DO AMARAL JÚNIOR (2012): Contribution of components of production on snap bean yield. *Crop Breed. Appl. Biot.* 12: 206-210.
- FEYZIAN, E., H. DEGHANI, A.M. REZAI, M. JALALI (2009): Correlation and sequential path model for some yield-related traits in melon (*Cucumis melo* L.). *J. Agric. Sci. Technol.* 11: 341-353.
- GIANNELOS, P.N., F. ZANNIKOS, S. STOURNAS, E. LOIS, G. ANASTOPOULOS (2002): Tobacco seed oil as an alternative diesel fuel: Physical and chemical properties. *Ind. Crop. Prod.* 16: 1-9.
- GOLDWASSER, Y., H. EIZENBERG, J. HERSHENHORN, D. PLAKHINE, T. BLUMENFELD, H. BUXBAUM, S. GOLAN, Y. KLEIFELD (2001): Control of *Orobanche aegyptiaca* and *O. ramosa* in potato. *Crop Prot.* 20: 403-410.
- GONÇALVES, M.C., A.M. CORREA, D. DESTRO, L.C.F. SOUZA, T.A. SOBRINHO (2003): Correlations and path analysis of common bean grain yield and its primary components. *Crop Breed. Appl. Biot.* 3: 217-222.
- HASANNEJAD, S., S.J. ZAD, H.M. ALIZADEH, H. RAHYMIAN (2006): The effects of *Fusarium oxysporum* on broomrape (*Orobanche aegyptiaca*) seed germination. *Commun. Agric. Appl. Biol. Sci.* 71: 1295-1299.
- HATAMI MALEKI, H., G. KARIMZADEH, R. DARVISHZADEH, A. SARRAFI (2011): Correlation and sequential path analysis of some agronomic traits in tobacco (*Nicotiana tabacum* L.) to improve dry leaf yield. *Aust. J. Crop Sci.* 5:1644-1648.
- IQBAL, M., M.A. ALI, A. ABBAS, M. ZULKIFFAL, M. ZEESHAN, H.A. SADAQAT (2009): Genetic behavior and impact of various quantitative traits on oil contents in sunflower under waters stress conditions at productive phase. *Plant Omics Journal* 2: 70-77.
- JOEL, D.M. (2000): The long-term approach to parasitic weeds control: Manipulation of specific developmental mechanisms of the parasite. *Crop Prot.* 19: 753-758.

- KATARINA, D., M. IVANA, F. KIRIL, S. OGNEN, D.E. GEORGI (2010): Genetic variability of Macedonian tobacco varieties determined by microsatellite marker analysis. *Diversity* 2: 439-449.
- KOZAK, M., M.S. KANG (2006): Note on modern path analysis in application to crop science. *Commun. Biometry Crop Sci.* 1: 32-34.
- LEILAH, A.A., S.A. AL-KHATEEB (2005): Yield analysis of canola (*Brassica napus* L.) using some statistical procedures. *Saudi J Bio Sci.* 12:103-113.
- LIQIN, L., H. YUBI, W. WEI, X. YALING (2012): Molecular cloning, sequence analysis and expression analysis of an NtWRKY6 from tobacco (*Nicotiana tabacum* L.) in abiotic stress. *American Journal of Plant Sciences* 3: 1520-1526.
- MARIAM, E.G., R. SUWANKETNIKOM (2004): Screening of tomato (*Lycopersicon esculentum* Mill.) varieties for resistance to branched broomrape (*Orobanche ramosa* L.). *Kasetsart J. (Nat. Sci.)* 38: 434 – 439.
- MARJANOVIC-JEROMELA A., R. MARINKOVIC, A. MIJIC, M. JANKULOVSKA, Z. ZDUNIC (2007): Interrelationship between oil yield and other quantitative traits in rapeseed (*Brassica napus* L.). *J. Central. Europ. Agr.* 8: 165-170.
- MOHAMMADI, S.A., B.M. PRASANNA, N.N. SINGH (2003): Sequential path model for determining interrelationships among grain yield and related characters in maize. *Crop Sci.* 43: 1690-1697.
- MOROZOV, I.V., C.L. FOY, J.H. WESTWOOD (2000): Small broomrape (*Orobanche minor*) and Egyptian broomrape (*Orobanche aegyptiaca*) parasitization of red clover (*Trifolium pratense*). *Weed Technol.* 14: 312-320.
- PÉREZ-DE-LUQUE, A., M.T. MORENO, D. RUBIALES (2008): Host plant resistance against broomrapes (*Orobanche* spp.): defence reactions and mechanisms of resistance. *Ann. Appl. Biol.* 152: 131–141.
- RADOJIĆIĆ, V., M. NIKOLIĆ, S. IĆTOVIĆ (2008): Some possibilities of tobacco waste utilization. *Ecological truth* 08, Proceedings of the VIII Scientific Professional Conference, Ecological Truth, Soko Banja, pp. 481-484.
- REN, N., M.P. TIMKO (2001): AFLP analysis of genetic polymorphism and evolutionary relationships among cultivated and wild *Nicotiana* species. *Genome* 44: 559-571.
- RUBIALES, D., C. ALCANTARA, A. PEREZ-DE-LUQUE, J. GIL, J.C. SILLERO (2003b): Infection by crenate broomrape (*Orobanche crenata*) in chickpea (*Cicer arietinum*) as influenced by sowing date, weather conditions and genetic resistance. *Agronomie* 23: 359–362.
- RUBIALES, D., C. ALCANTARA, J.C. SILLERO (2003a): Variation in resistance to *Orobanche crenata* in species of *Cicer*. *Weed Res.* 44: 27–32.
- SABAGHNI, N., H. DEGHANI, B. ALIZADEH, M. MOGHADDAM (2010): Interrelationships between seed yield and 20 related traits of 49 canola (*Brassica napus* L.) genotypes in normal and water-stressed environments. *Span J. Agric. Res.* 8: 356-370.
- TUNCTURK, M., V. ÇİFTÇİ (2005): Selection criteria for potato breeding. *Asian J. Plant Sci.* 4: 27–30.
- WANG, G., M.S. KANG, O. MORENO (1999): Genetic analyses of grain-filling rate and duration in maize. *Field Crops Res.* 61:211-222.
- YASIN, A.B., S. SINGH (2010): Correlation and path coefficient analyses in sunflower. *J. Plant Breed. Sci.* 2: 129-133.

**SEKVENCI PAT ANALIZA U ODREĐIVAJU INTERRELACIJE PRINOSA I
KOMPONENATA PRINOSA KOD DUVANA (*Nicotiana tabacum* L.) U NORMALNIM I
USLOVIMA STRESA**

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Izvod

Kod različitih genotipova duvana vršena su merenja odnosa prinosa i osobina: datum cvetanja (FD) visina biljaka (PH), broj listova (NL), indeks površine lista (LAI), težina svežeg lista (FWL), težina svežeg korena (FWR), težina suve supstance korena (DWR), površina svežeg lista bez težine lista (APFW), biomasa (BIO), žetveni indeks (HI), prinos suvih listova po biljci (DLYP) u normalnim uslovima gajenja uslovima biološkog stresa (zakorovljenost sa *Orobanche aegyptiaca*) u Urmia centru za duvan u Iranu u toku 2006 – 2009. Na osnovu dobijenih rezultata osobine biomasa (BIO) APDW i DWR su utvrđene kao najznačajniji faktori kako u normalnim tako i u uslovima stresa za programe oplemenjivanje duvana na povećan prinos.

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