DOI: http://dx.doi.org/10.22092/cbj.2012.100419

Analysis of genetic variation for morphological and agronomic traits in Iranian oriental tobacco (*Nicotiana tabaccum* L.) genotypes

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Received: July 2011

ABSTRACT

Darvishzadeh, R. and H. Hatami Maleki. Analysis of genetic variation for morphological and agronomic traits in Iranian oriental tobacco (*Nicotiana tabaccum* L.) genotypes. **Crop Breeding Journal 2(1):57-61.**

To assess the genetic diversity for morphological and agronomic traits in Iranian oriental tobacco germplasm, 100 oriental and semi-oriental tobacco genotypes from the Urmieh Tobacco Research Center collection were evaluated in the field using a simple square lattice design with two replications. Some agronomic traits were measured on five randomly selected plants in each plot. Genotypes were clustered into similar groups using UPGMA based on squared Euclidean distance after data normalization. Four distinct groups were established. Discrimination function analysis performed to determine the accuracy of the grouping developed by cluster analysis showed that the total success rate of grouping was 97%. Among developed groups, groups 1 and 4 had the maximum Mahalanobis distance value of 67.4. Results revealed that the first three discriminant functions were significant, and their standardized discrimination function coefficients indicated that among the studied traits, days to 50% flowering, dry leaf yield and number of leaves per plant were the most important for discriminating among tobacco genotypes.

Key words: Cluster analysis, discriminant analysis, genetic diversity, Mayhalanobis distance, leaf yield.

INTRODUCTION

Tobacco (*Nicotiana tabaccum* L.) is a member of the nightshade (Solanaceae) family, the largest and most diverse family within the angiosperms, for it harbors 3,000-4,000 species (Olmstead *et al*., 2008), of which a considerable number are of major economic importance as crop, vegetable or ornamental species throughout the world. The genus *Nicotiana* consists of nearly 100 species mainly from tropical and subtropical America; almost all commercial tobacco belongs to the *tabaccum* species (Narayan, 1987; Ren and Timko, 2001). Several types of *N. tabaccum* are defined to a large extent by region of production, projected use in manufacturing, method of curing (flue-, air-, sun- and fire-cured tobacco), as well as their 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, small-leafed type which is grown in Iran, Turkey, Greece, Bulgaria, Lebanon and the Republic of Macedonia. It can be grown on low fertility soils (Chaplin, 1975; Davis and Nielsen, 1999). To produce an American blend type of cigarette, oriental tobacco is mixed with more robust tobaccos such as Virginia and Burley.

Estimating genetic diversity and determining the relationships among germplasm collections enhances the efficiency of germplasm collection management (Nisar *et al.,* 2008) and genetic improvement (Geleta *et al.,* 2005). When developing breeding populations, plant breeders may use data on genetic similarity to complement phenotypic information (Nienhuis *et al.,* 1993; Greene *et al.,* 2004; Yuzbasioglu *et al.,* 2006). The future of successful breeding programs depends on the availability of genetic variability to increase productivity. Morphological characterization is the first step in describing and classifying germplasm collections (Smith *et al.,* 1991). Morphological characteristics are the strongest determinants of the agronomic value and taxonomic classification of plants. Compared with other approaches, morphological evaluation is direct, inexpensive and easy. If there is an adequately large sample size and significant variation in measured traits, phenotypic variation can provide a reasonable representation of overall genetic performance (Humphreys, 1991).

Multivariate statistical techniques that simultaneously analyze multiple measurements on each individual under investigation will be

advantageous in analyzing genetic diversity and classifying germplasm collections (Mohammadi and Prasanna, 2003). Recently, several studies have been conducted to assess the genetic diversity of many plants based on morphological traits and using multivariate procedures including principal component analysis (wheat: Hailu *et al.,* 2006; tobacco: Zeba and Isbat, 2011; festuca: Afkar *et al.,* 2009; peach: Nikolic *et al.,* 2010), cluster analysis (heiry vetch: Yeater *et al.,* 2004; wheat: Hailu *et al.,* 2006; festuca: Afkar *et al.,* 2009; peach: Nikolic *et al.,* 2010; tobacco: Zeba and Isbat, 2011), and discriminant analysis (tall fescue: Vaylay *et al.,* 2002; peanut: Safari *et al.,* 2008). This provided proof that cluster analysis can be used for genetic diversity evaluation and germplasm classification (Yeater *et al.,* 2004; Safari *et al.,* 2008). Discriminant function analysis as a post-cluster analysis method was able to recognize the accuracy of clustering when used by several researchers (Safari *et al.,* 2008; Rahimi *et al.,* 2009). The magnitude of each trait in the genetic diversity of tall fescue (Vaylay *et al.,* 2002), hairy vetch (Yeater *et al.,* 2004) and peanut (Safari *et al.,* 2008) was identified using discriminant functions.

Due to its geographical situation, northwestern Iran is one of the most favorable regions for growing oriental tobacco. Little is known about the genetic variability of available oriental tobacco germplasm. This study was conducted to investigate the genetic diversity of oriental tobacco germplasm based on morphological and agronomic traits for use in tobacco breeding programs aimed at developing high yielding tobacco cultivars.

MATERIALS AND METHODS

One hundred oriental and semi-oriental tobacco genotypes were evaluated at the Urmieh Tobacco Research Centre (UTRC) (Table 1). The 'SPT' lines known as Chopogh tobacco are inbred lines selected by the UTRC from local landraces using the single-plant or pure-line selection method (Table 1). The 'PD' lines are recombinant inbred lines (RILs) derived from a cross between Basma S. 31 and Dubec 566 made in the UTRC. Jahrom lines are Iranian water-pipe tobacco lines selected from local landraces by single-plant or pure-line selection method in the UTRC. Also used in this study were inbred lines originating from different countries and introduced from CORESTA (Cooperation Center for Scientific Research Relative to Tobacco, Paris, France) collections or pure lines kindly provided by Iran's Tirtash Tobacco Research Centre.

The experimental design used was a simple

Table 1. Name and codes of oriental tobacco genotypes.

No.	Genotype	No.	Genotype	No.	Genotype
1	TR1	35	PZ17	69	SPT406
2	TR21	36	PD 205 (Urmia 205)	70	SPT408
3	TR93	37	PD 209 (Urmia 209)	71	SPT409
4	ch.T 269-12	38	PD 379 (Urmia 379)	72	SPT410
5	TS8	39	Erzeogovina	73	SPT412
6	FK 40-1	40	PBD 6 X Mut 4	74	SPT413
7	Jahrom 12	41	SPT 414XPobeda	75	SPT420
8	Samson1	42	Trabzon	76	SPT430
9	Samsoun 959	43	Line 20	77	SPT432
10	Samson Katerini	44	Jahrom 14	78	SPT433
11	Samsoun Dere	45	ch.Trabzon 269-12B	79	SPT434
12	TYK-Kula	46	KP14/a	80	SPT436
13	Alborz 23	47	Nevrokop	81	SPT439
14	SS289-2	48	Mutant No2	82	SPT441
15	Basma 12-2	49	Mutant No3	83	ʿPD324
16	Basma16-10	50	Jahrom 15	84	PD325
17	Basma 104-1	51	L16	85	PD328
18	Basma 181-8	52	ch.t 209-12e	86	PD329
19	Basma Mahalades	53	Xanthi	87	PD336
20	KB	54	ch.T.283-8	88	PD345
21	GD165	55	TK28	89	PD364
22	D566	56	ch.T.266-6	90	PD371
23	Pobeda 2	57	Trabzon No 23	91	PD371
24	Pobeda 3	58	CH.T.273.38	92	PD381
25	Krumovgrad 42	59	Pobeda 1	93	Mutant 4
26	Krumovgrad N.H.H.659	60	PL7	94	CHT269-12XFK401
27	Harmanli 11	61	L17	95	TK FK40-1
28	Immuni 3000	62	Melnik 261	96	TB22
29	Kharmanli 163	63	KB101	97	Krumovgrad
30	Izmir	64	Trabzon H.T.1	98	Krumovgrad kanti
31	Kuklen 6	65	Trimph	99	Ohdaruma
32	Nevrokop 261	66	Basma S.31	100	Matianus
33	Ploudiv	67	SPT403		
34	TK23	68	SPT405		

square lattice with two replications. Each plot consisted of three lines, 5 m long, with approximately 8,000 plants ha^{-1}. The seed was sown at a rate of approximately 5 g m^{-2} and covered with a fine layer of well fermented and sieved sheep manure. Just before transplanting, the field was thoroughly plowed and disked to break up the soil and smooth and level the field. Tobacco seedlings were transplanted to the plots when average plant height was about 12 cm. Three weeks after transplanting, once the plants were well established, a short-handled hoe was used to loosen the soil around each plant and remove weeds. Field irrigation was carried out after 80% depletion of soil available water (Salehzadeh *et al.,* 2009). The plants were not topped as is common with most other types of tobacco, such as Virginia and Burley. Agronomic traits including plant height (PH), stem girth (SG), leaf number (LN), leaf length (LL), leaf width (LW) and days to 50% flowering (D50F) were measured on five randomly selected plants in each plot (Kara and Esendal, 1995). Dry leaf yield and fresh leaf yield were assessed using all plants in the plots, except for those on the borders.

A normality test of recorded data was performed, and an analysis of variance was carried out using the general linear model (GLM) procedure in SAS software (SAS Institute Inc.). Genotypes were clustered using UPGMA clustering criterion with squared Euclidean distance after data normalization. Discriminant function analysis was then performed to determine the accuracy of grouping. The difference between centroid values of the resulting groups was estimated using the Mahalanobis distance (D^2) calculated as:

$$D^2 = \left(\overline{X}_1 - \overline{X}_2\right)' S^{-1} \left(\overline{X}_1 - \overline{X}_2\right)$$

where S^{-1} is the inverse of the pooled sample variance-covariance matrix, and $\overline{X}_1$ and $\overline{X}_2$ are the respective vectors of measurements in groups 1 and 2. Standardized coefficients of significant discriminant functions were used as criteria for determining the magnitude of the variation of each trait in oriental tobacco germplasm.

RESULTS AND DISCUSSION

The analysis of variance revealed significant differences among oriental tobacco genotypes based on agronomic traits (data not shown). Results were in accordance with findings of previous studies on other types of tobacco using morphological and agronomic attributes (Wenping *et al.,* 2009; Zeba and Isbat, 2011). Using the UPGMA hierarchical algorithm on a squared Euclidean distance matrix, oriental tobacco genotypes were classified into four separate groups (Table 2). Group 1 consisted of genotypes or lines known as Chopogh tobaccos, which can be distinguished from other types by their dwarf plant height. Sixty-two percent of genotypes included in group 2 are known as Basma tobaccos (Table 2). These tobaccos are oriental form of oriental tobacco genotypes. Tobacco genotypes known as Trabozon were located in group 3 (Table 2). These tobaccos are considered semi-oriental tobacco genotypes because their morphological traits are intermediate between oriental and western tobacco types. Among tobacco genotypes, Ohdaruma and Trimph had morphological characteristics different from those of other genotypes and are located in group 4 (Table 2). These genotypes resemble Virginia type tobaccos. The accuracy of the groups produced was reassessed using discriminant function analysis (Table 3). The total success rate of the discriminant function was 97% (Table 3), which indicates it was successful in discriminating different groups. This result is supported by the findings of other researchers such as Balochi *et al.* (2001) in barley and Janes *et al.* (2003) in corn. Moreda *et al.* (2003) grouped 85 tea samples into Asian and African groups with cluster analysis using Ward's minimum variance method and squared Euclidean distance; discriminant

Table 2. Grouping of oriental tobacco genotypes based on the UPGMA algorithm.

Group	Genotypes
1	**TK23, PZ17, TR93, urmia 205, ch.T 269-12, urmia 209, TS8, urmia 379, FK 40-1, Erzeogovina, PBD 6 X Mut 4, Samson1, SPT 414Xpobeda, Samsoun 959, Trabzon, Line 20, Samsoun Dere, ch.Trabzon 269-12B, Alborz 23, SS289-2, Nevrokop, Basma 12-2, Mutant No2, Basma16-10, Mutant No3, Basma 104-1, PD324, Basma 181-8, L16, PD325, ch.t 209-12e, PD328, KB, PD329, ch.T.283-8, PD336, D566, TK28, PD345, Pobeda 2, ch.T.266-6, PD364, Pobeda 3, Trabzon No 23, PD371, Krumovgrad 42, CH.T.273.38, PD371, Pobeda 1, PD381, Harmanli 11, PL7, Mutant 4, Immuni 3000, L17, CHT269-12XFK401, Kharmanli 163, Melnik 261, TK, FK40-1, KB101, TB22, Kuklen 6, Trabzon H.T.1, Krumovgrad, Nevrokop 261, Krumovgrad kanti, loudiv, Basma S.31**
2	**Ch.T 269-12, Samson1, Kuklen 6, ch.Trabzon 269-12B, ch.t 209-12e, KB101**
3	**SPT432, SPT433, SPT434, SPT436, SPT439, SPT441, SPT413, SPT420, SPT430,SPT403, SPT405, SPT406, SPT408, SPT409, SPT410, SPT412, TYK-Kula, Basma Mahalades, Izmir, Xanthi, TR1, TR21, Samson Katerini, Jahrom 12, GD165, Krumovgrad N.H.H.659, KP14/a, Jahrom 50, Jahrom 14**
4	**Ohdaruma, Trimph**

Table 3. Determination of clustering accuracy using discriminant function analysis.

		Predicted group membership				Total
		1	2	3	4	
Original	Count	27	2	0	0	29
		1	61	0	0	62
		0	0	5	1	6
		0	0	0	2	2
	Percentage	93.1	6.9	0	0	100
		1.6	98.4	0	0	100
		0	0	83.3	16.7	100
		0	0	0	100	100

97% of the original groups were correctly classified.

function analysis revealed that 94.4% of the grouping was correct.

The separation of the groups was measured using the Mahalanobis distance (Table 4). All groups had significant pair-wise distances and the maximum value was observed between group 1 and group 4. However, this is in contrast with the results of Yang *et al.* (2007), who found low genetic diversity in oriental tobacco using inter simple sequence repeat markers.

Table 4. Pairwise squared distances between clustered groups calculated by Mahalanobis distance (D^2).

	Group 1	Group 2	Group 3
Group 2	10.9**		
Group 3	66.8**	38.4**	
Group 4	67.4**	49.5**	33.9**

Significant discriminant functions and their standardized coefficients could appropriately be applied to interpret genetic diversity among oriental tobacco genotypes (Table 5). Similar results have also been reported by Yeater *et al.* (2004) and Safari *et al.* (2008) in hairy vetch and peanut, respectively. Indeed, these coefficients reflect the common variance of measured characters with functions that could be beneficial in assessing the relative contribution of each variable to each discriminant function (Cruz-Astillo *et al.*, 1994). In the first function, high coefficients were observed for days to 50% flowering (D50F) and dry leaf yield (DLY) (Table 5). In the second function, however, leaf number (LN) had high coefficient value. In the third function, component DLY had higher loading next to D50F. Therefore, these characters could have effective roles in developing variation among oriental tobacco genotypes. These findings are in agreement with reports by Hatami Maleki *et al.* (2011), who used sequential path analysis and showed that leaf number and days to 50% flowering are important characters for developing oriental tobacco cultivars with high dry leaf yield.

Table 5. Standardized discriminant function coefficients of measured characters in oriental tobacco genotypes.

Character	Function		
	1	2	3
No. of leaves per plant	0.29	-0.61	-0.52
Leaf length	-0.19	-0.09	-0.26
Leaf width	-0.04	0.37	-0.21
Fresh leaf yield	-0.46	-0.41	0.41
Dry leaf yield	1.02	0.35	-1.09
Plant height	-0.18	-0.51	-0.04
Stem girth	0.21	0.50	0.14
Days to 50% flowering	0.61	0.21	0.88

CONCLUSION

In this study, large genetic diversity was found in oriental tobacco germplasm of Iran based on morphological and agronomic characters. Oriental tobacco genotypes were classified into four different groups. Discriminant function analysis determined the accuracy of grouping. The maximum Mahalanobis distance was observed between group 1 and group 4. This information could be used as criteria for identifying parents with large differences to be utilized in building mapping populations as well as in hybrid breeding programs. Standardized discriminant function coefficients could be used to identify important traits causing variation in oriental tobacco genotypes. Days to 50% flowering, dry leaf yield and number of leaves per plant made significant contributions to observed genetic diversity in oriental tobacco germplasm.

ACKNOWLEDGMENTS

The authors are grateful to Dr. Iraj Bernousi and Dr. Morad Jafari from the Faculty of Agriculture, Urmieh University, Urmieh, Iran, for critically reading the manuscript and making valuable suggestions to improve it. The authors would also like to thank the Institute of Biotechnology, Urmieh University, Urmieh, Iran, as well as Urmieh Tobacco Research Centre, for their financial support.

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