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GE Interaction and Stability Analysis in Some Basma Type Oriental Tobacco (*Nicotiana tabacum* L.) Lines

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ABSTRACT

Turkey has long been the leader in oriental tobacco (*Nicotiana tabacum* L.) production in the world. Standard cultivars are needed to increase the yield and quality of tobacco production. This study aimed to determine the most stable cultivar candidates by evaluating the performances of tobacco genotypes grown in different environmental conditions. Field trials were carried out in Bafra district of Samsun Province, the district with most tobacco production in Mid-Black Sea Region, and Evciler, Karayaka and Gümüşhacıköy where the Basma type oriental tobaccos are produced. The experimental design was randomized complete blocks with three replications in 2017. The study material consisted of 21 lines selected by morphological characteristics and identified by DNA fingerprinting analysis and four standard cultivars/lines. Chemical analyses were carried out using the HPLC method. The stability of genotypes was determined by regression coefficient (b_i), regression constant (a), determination coefficient (r^2), coefficient of variation (CV) and deviation from regression (S^2d) parameters using the leaf yield, quality grade index, nicotine and sugar content values. The ERB-6, ERB-7, ERB-11, ERB-13, ERB-16, ERB-18, ERB-21 and ERB-30 lines were considered the prominent candidates based on the stability parameters and other traits investigated. Therefore, future studies should be continued using the aforementioned lines. In conclusion, much more detailed studies are needed on hopeful cultivar candidates determined as stable for production areas of the Basma type oriental tobacco.

Keywords: Adaptability, HPLC, Nicotine, Quality, Sugars, Yield

1. Introduction

Turkey is the largest (30%) known producer of high-quality aromatic oriental tobacco (*Nicotiana tabacum* L.). It produces 93.665 tons of leaf tobacco in 99.528 ha areas with 64.541 farmers in 25 provinces, 98 districts and 1.942 villages in Turkey, and its production that a total value of approximately 250 million dollars is made 66% in the Aegean, 15% in Southeast Anatolia and 10% in Black Sea region, the remaining 9% are made in Marmara, Eastern Anatolia and Mediterranean regions according to Republic of Turkey Ministry of Agriculture and Forestry Statistics (Yilmaz et al. 2020). The oriental tobacco has sufficient sugar content with less carcinogen and nicotine, as well as a more favorable aroma than the other tobacco types. Therefore, oriental tobacco is often blended with Virginia and Burley tobaccos, which have a stronger effect, and used in American blend type cigarettes (Darvishzadeh et al. 2013). In this respect, tobacco must be regularly analyzed and screened for proper use. Alkaloids are important components of tobacco and have a marked influence on tobacco quality and consumption (Andersen et al. 1991). nicotine is the most abundant alkaloid among more than the (20) alkaloids in tobacco and causes extensive consumption of tobacco products worldwide (Xia et al. 2014). Sugars are the primary metabolites that contribute to the growth of tobacco plants (Cai et al. 2015). Sugar composition is directly related to the taste and aroma of the tobacco (Nagai et al. 2012). The amount of sugar in tobacco types is quite variable and primarily depends on the curing process. Glucose and fructose, called reducing sugars, are the most important of soluble sugars (Leffingwell 2001; Roemer et al. 2012). Therefore, the chemical structure should be reliably determined in defining leaf tobacco for blenders and determining potential toxicity in terms of health (Cai et al. 2015).

Adaptation strength and stability of the varieties at different districts are very important for breeders to ensure optimum yield. The stability, as well as agronomic, morphological, pathological and technological traits (Zencirci et al. 1990), and the initiative of the breeder (Keser et al. 1999) should be considered in line selection. The characteristics of tobacco genotypes as in all plants are the consequence of the common effect of the genotype (G), environment (E) and GE interaction (Ekren & Sekin 2008).

Genotypes tested in different environments show variations in yield and other characteristics due to environmental factors such as soil, climate and presence of disease pathogens. These fluctuations are often attributed to the effect of GE interactions, which is widely used in many plants. Many researchers use the terms of “stability” and “compatibility” to consistently address the high genotype efficiency in different environments (Romagosa & Fox 1993). Lin & Binns (1994) have identified two types of stable genotypes, with stable performance in all environments and high performance in specific environments (with specific adaptation).

The GE interactions of genotypes can be determined by classical statistical analyzes, which do not provide information about the stability of genotypes (Kilic et al. 2003). Therefore, various statistical methods have been proposed to assess the stability of genotypes in changing environments. Dehghani et al. (2006) emphasized that a single method cannot adequately explain the efficiency of genotypes in different environments. The most commonly used approach is based on a linear regression of genotype yield on an environmental index derived from the average performance of all genotypes in each environment (Finlay & Wilkinson 1963). This model provides two stability parameters. The first one is the mean linear regression coefficient (b_i) of the genotypes in the environmental index and the second one is a deviation from regression (S^2d) for each genotype. The richness of origin in the region is controlled by the tobacco producers due to the exchange of seeds and seedlings. Mixed cultivation of the different tobacco types restrains obtaining the product with the desired characteristics. Breeding studies are needed to develop high-yield and high-quality varieties to eliminate the problems arising from especially in terms of yield and quality. In this study, field experiments have been carried out using (21) the Basma type tobacco lines, which have some prominent characteristics, in four districts where the most common tobacco production takes place to determine the performance and the stabilities.

2. Material and Methods

2.1. Material

Cultivation areas of the Basma tobacco (*Nicotiana tabacum* L.) types in Turkey (especially in Tokat province) have been screened in 2015, morphologically different plants were identified and seeds were collected. The material of the study composed of (25) tobacco genotypes including (21) Basma type tobacco lines identified as the Basma type in Turkey by Kurt (2019) and (4) standard tobacco varieties/lines.

2.2. Field Experiments

Seedlings of the 25 genotypes were grown in a float system with peat medium within foam viols. Composite fertilizer containing 20.10.20 (N, P, K) + micronutrients (Iron 0.4%, Manganese 0.4% and Zinc 0.4%) was mixed with 500 g t^{-1} water in float pond water to supply nutrients for the seedlings. The experimental fields were chosen from different altitudes where the study material could be cultivated. Field studies were carried out in the Evciler (40°36'43.48" N, 36°36'5.25" E, 581 m) and Karayaka (40°44'16.45" N, 36°33'58.31" E, 302 m) villages of Tokat-Erbaa, in Samsun-Bafra (41°33'45.29" N, 35°52'18.35" E, 26 m) and Amasya-Gümüşhacıköy (40°53'1.03" N, 35°12'47.98" E, 848 m) districts in 2017. Before the planting of seedlings, 60 kg ha^{-1} N, 40 kg ha^{-1} P_2O_5 and 60 kg ha^{-1} K_2O were applied to the experimental fields (Yilmaz & Kinay 2011). The field experiments were carried out in a randomized block design with three replications. The seedlings were planted with 45 cm inter-row and 12 cm intra-row spacings on 5 m long plots. The seedling planting was performed on May 21, 2017, in Evciler, May 19, 2017, in Karayaka, July 4, 2017, in Bafra and June 29, 2017, in Gümüşhacıköy.

Table 1- Soil analysis results

Properties	*Districts*			
	Evciler	*Karayaka*	*Gümüşhacıköy*	*Bafra*
P_2O_5 (kg da^{-1})	5.13 Low	6.18 Modarate	4.85 Low	3.45 Low
K_2O (kg da^{-1})	169.70 High	175.30 High	156.80 High	137.17 High
Lime (%)	10.2 Modarate calca.	2.39 Calcareous	5.17 Modarate calca.	12.73 Modarate calca.
Org. Mat. (%)	0.95 Very low	1.43 Low	2.36 Modarate	1.76 Low
pH	7.99 Slightly alkaline	7.81 Slightly alkaline	7.98 Slightly alkaline	7.61 Slightly alkaline
EC (dS m^{-1})	0.25 Very low	0.13 Very low	1.12 Very low	0.72 Very low
Texture	Clay loam	Sandy loam	Sandy loam	Sandy loam

The soil in Evciler experimental field had clay loam texture and the other three districts had sandy loam texture. Electrical conductivity indicated a very low salinity level in all experimental fields. The soils were slightly alkaline and the highest organic matter content was recorded in the Gümüşhacıköy. The experimental fields in Bafra and Karayaka fields had low and Evciler had very low organic matter content. The soils in Evciler, Gümüşhacıköy and Bafra districts were moderately calcareous while in the Karayaka was calcareous. The potassium contents of all experimental fields were high. Plant available phosphorus content of an experimental field in Karakaya was moderate while the other three fields had low in phosphorus content (Table 1). The temperature values during the seven months covering the seedling, field and curing periods of tobacco were similar to the long-term averages, while the relative humidity values were higher than the long-term averages. The average relative humidity during this period was 69.86% in Erbaa, 64.43% in Gümüşhacıköy and 82.24% in Bafra. The total precipitation during the vegetation period was 222.2 mm in Erbaa, 256.5 mm in Gümüşhacıköy and 222.1 mm in Bafra. The precipitation was 36.1 mm lower in Erbaa, 47.1 mm lower in Gümüşhacıköy and 152.2 mm lower in Bafra compared to the long-term averages.

2.3. Investigated Parameters

A genotype is defined as stable when an S^2d value is close to zero, a b_i value is close to 1.0 and mean value (X_{mean}) is higher than the overall mean value (Eberhart & Russell 1966). A b_i value higher than 1.0 indicates the adaptation to a good environment, and a b_i value lower than 1.0 indicates the adaptation to a poor environment. The low S^2d values indicate that genotypes are not affected by the changing environmental conditions (Albayrak et al. 2005). The coefficient of variation (CV) for genotypes in different environments can be used as a stability parameter (Francis & Kannenberg 1978). Positive and high-value of regression constant (a), which represents the first point in the regression line (Finlay & Wilkinson 1963) indicates the high efficiency of a genotype in poor environmental conditions. Therefore, a stable genotype is expected to have a positive high constant value and a high coefficient of determination (r^2) (Eberhart & Russell 1966). The adaptation of genotypes was separated into nine regions using the general experimental mean, regression coefficient and confidence limits (Confidence interval = $X_{mean} \pm t\alpha.Sx$) in terms of the parameter considered (Uzun et al. 2012) (Figure 1). The significance of genotype x environment interactions and stability of genotypes in yield, quality grade index, nicotine and reducing sugar characteristics were evaluated by analysis of variance (ANOVA) using SAS 9.0 software.

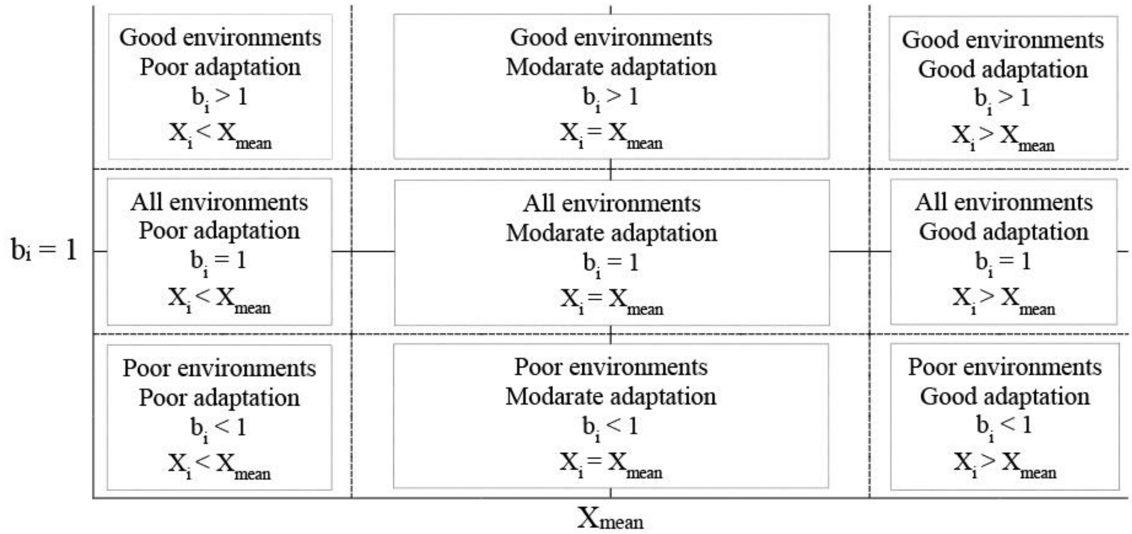

Figure 1- Mathematical and verbal expression of genotypic adaptation regions

Yield and quality grade index: All leaves harvested from the experimental plots in 3 different periods (Table 2) were dried by the sun-cured method, the moisture content was fixed to 17% and calculated as kg da^{-1}. The yield values were determined according to the American Grading method in dried leaf samples of each plot.

Table 2- Priming dates

Priming	***Districts***			
	Evciler	*Karayaka*	*Gümüşhacıköy*	*Bafra*
1. priming	03.07.2017	27.06.2017	03.08.2017	15.08.2017
2. priming	18.07.2017	11.07.2017	23.08.2017	04.09.2017
3. priming	12.08.2017	08.08.2017	23.09.2017	26.09.2017

Chemical analysis: Nicotine, glucose and fructose contents were determined by using HPLC and the sum of (glucose + fructose) was evaluated as reducing sugar. In the nicotine analysis (%), moisture-free tobacco samples were ground, 200 mg of powdered samples were put into 50 mL falcon tubes, 1% Acetic acid and Acetonitrile were added and left in the ultrasonic water bath for 30 minutes (Kinay 2018; Kurt 2019). Samples removed from the water bath were centrifuged at 4000 rpm for 10 minutes. The supernatant remaining above the precipitated sample was removed by an injection. The solution was passed through the filter (Nylon 0.45 µm) and placed into the vial with the sample code written. The extracts were analyzed by HPLC with a diode array detector at a flow rate of 1 mL min^{-1} and a column temperature of 35 °C with a C18 column (Moghbel et al. 2015). For glucose and fructose analysis (percentage), one gram of powdered sample was weighed into the falcon tubes and (1%) acetic acid and methanol were added. After the addition of solvents, they were mixed and placed in an ultrasonic water bath. The ultrasonic water bath was allowed to stand for 30 minutes and the samples removed from the water bath were centrifuged at 4000 rpm for eight minutes. The supernatant above the precipitated sample was removed by an injection. The removed solution was passed through the filter (Nylon 0.45 µm) and placed into the vial with the sample code written. The solution was analyzed using an HPLC with a refractive index detector in the carbohydrate column with 1.5 mL flow and column temperature at 40 °C (Nagai et al. 2012). The peaks obtained from the sample chromatograms were identified by comparing the peaks obtained from the standards and peak areas were calculated according to their standard calibrations (r^2; 0.999 and 1.0). Extraction recovery ratios indicating the reliability of the analyzes were 101% in nicotine, 106% in glucose and 102% in fructose (Kinay 2018; Kurt 2019).

3. Results and Discussion

3.1. Yield

The results of variance analysis for the yield were given in Table 3, and the results of the stability parameters related to yield were presented in Table 4. The graph for adaptation classes determined using the regression coefficients and the mean yield values were shown in Figure 2. The mean yield of the experiment was 178.72 kg da^{-1}. High yield is a desired characteristic; thus, genotypes with higher yield values than average yield were considered to meet the first condition of the stability. The confidence interval for the regression coefficients of the genotypes was ±0.11, and the confidence interval for the mean yield was calculated as ±5.41, and the lower and upper limits were given in Figure 2. The genotypes with b_i values between 0.89 and 1.11 indicated a stable performance in all environments and those with b_i values lower or higher than these values pointed out the stable performance in certain environments. The ERB-16, ERB-18 and ERB-30 lines, which had a yield above the average and a b_i value equal or close to 1.0, were classified as stable in all environments. The ERB-16 line was stable with a positive regression constant (21.67) and a low coefficient of variation (9.17) value. However, the r^2 value of this line is expected to be higher than 0.88, and the S^2d value is expected to be lower than 309.12 to meet all the conditions of the stability. Similarly, the ERB-18 line had a regression constant (-4.04) closest to the positive, despite the high r^2 (0.98), low CV (4.06) and low S^2d (57.87) values. In this group, the ERB-30 line was a stable genotype with positive regression constant (36.74), high r^2 (0.97), low CV (3.83) and low S^2d (56.08) values in addition to a bi value in high yield and confidence interval (Table 4; Figure 2).

Table 3- The results of the analysis of variance

Variation sources	*D.F.*	*Yield*	*Quality Grade Index*	*Nicotine*	*Reducing sugar*
Environment (E)	3	133967.78**	17598.68**	34.37**	25.17**
Genotype (G)	24	2063.72**	939.18**	0.32**	12.30**
G x E	72	908.22**	177.89**	0.30**	9.55**
Error	198	409.34	53.43	0.05	0.43
C.V. (%)		11.32	10.46	21.53	7.70

D.F.; The degree of freedom, C.V.; Coefficient of variation, **; Indicates the significance level at P<0.01

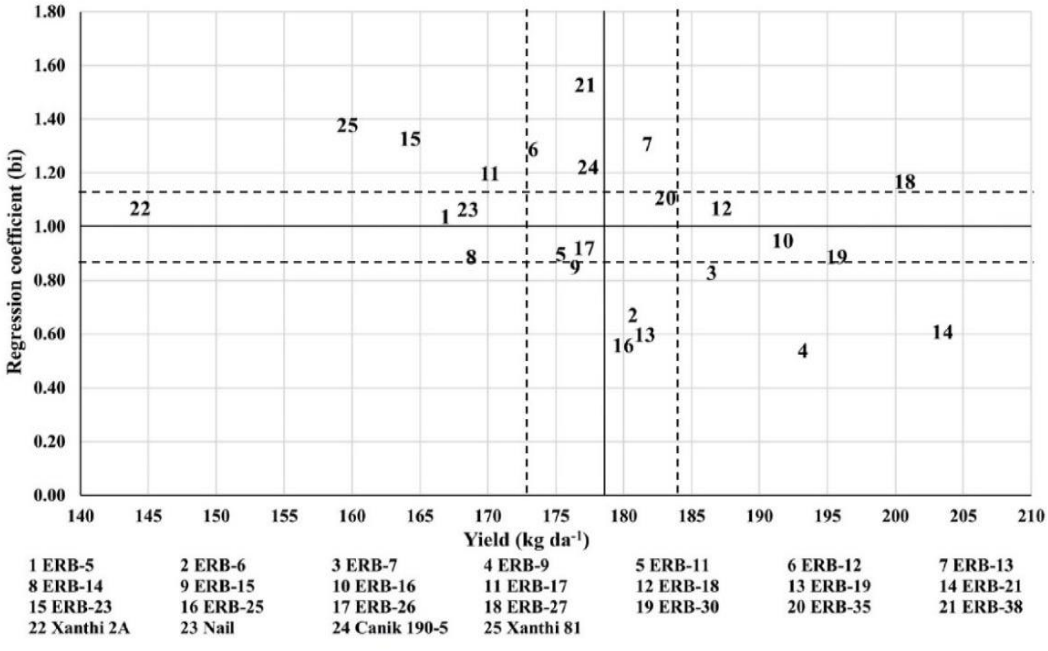

Figure 2- Stability conditions of different tobacco genotypes according to yield and the regression coefficient

Table 4- Values of the stability parameters for yield and quality grade index

No	Genotypes	Yield (kg da^{-1})						Quality grade index (%)					
		X_{mean}	b_i	a	r^2	CV	S^2d	X_{mean}	b_i	a	r^2	CV	S^2d
1	**ERB-5**	167.95	1.02	-13.84	0.96	6.36	114.02	76.34	0.73	24.64	0.67	12.58	92.17
2	**ERB-6**	180.82	0.66	63.24	0.73	11.42	426.61	72.34	1.05	-1.13	0.94	6.37	21.21
3	**ERB-7**	186.50	0.83	38.00	0.98	2.50	21.73	78.03	0.91	14.07	0.94	5.40	17.78
4	**ERB-9**	193.20	0.54	97.35	0.96	2.92	31.90	56.92	1.07	-18.12	0.75	20.34	134.07
5	**ERB-11**	175.80	0.90	14.63	0.99	1.35	5.65	57.58	0.92	-6.49	0.75	17.21	98.23
6	**ERB-12**	173.35	1.29	-58.12	0.99	3.76	42.60	71.55	1.11	-6.12	0.93	8.04	33.09
7	**ERB-13**	181.74	1.31	-53.28	0.92	11.03	401.99	78.65	0.88	16.77	0.88	7.78	37.48
8	**ERB-14**	168.79	0.89	8.54	0.95	5.75	94.13	67.44	0.95	1.29	0.79	13.60	84.18
9	**ERB-15**	176.48	0.87	20.93	0.70	16.46	843.52	77.76	1.16	-3.91	0.82	13.40	108.50
10	**ERB-16**	191.74	0.95	21.67	0.88	9.17	309.12	67.01	1.29	-23.68	0.90	12.39	68.94
11	**ERB-17**	170.19	1.20	-44.56	0.94	8.67	218.08	60.20	1.05	-13.24	0.80	16.58	99.62
12	**ERB-18**	187.24	1.07	-4.04	0.98	4.06	57.87	55.90	1.14	-23.84	0.93	10.64	35.36
13	**ERB-19**	181.33	0.60	73.50	0.78	8.94	263.07	79.70	0.85	20.49	0.74	11.96	90.88
14	**ERB-21**	203.52	0.61	94.23	0.99	0.86	3.08	72.62	0.57	32.51	0.51	14.68	113.68
15	**ERB-23**	164.31	1.33	-73.81	0.93	10.79	314.50	73.34	0.78	18.81	0.67	13.90	103.97
16	**ERB-25**	179.99	0.56	79.89	0.73	9.64	301.44	59.78	0.86	-0.48	0.99	1.75	1.09
17	**ERB-26**	177.30	0.91	15.25	0.94	6.68	140.25	69.97	1.19	-13.29	0.79	16.32	130.36
18	**ERB-27**	200.72	1.17	-7.86	0.85	12.48	627.60	46.73	1.40	-51.47	0.98	7.03	10.79
19	**ERB-30**	195.72	0.89	36.74	0.97	3.83	56.08	75.56	1.17	-6.54	0.77	15.98	145.85
20	**ERB-35**	183.11	1.11	-15.68	0.87	12.13	492.99	75.39	0.65	30.20	0.55	14.47	118.92
21	**ERB-38**	177.96	1.53	-94.80	0.95	9.98	315.60	78.09	1.13	-0.80	0.92	8.07	39.67
22	**Xanthi 2A**	144.45	1.07	-47.61	0.99	2.37	11.73	77.07	1.20	-7.12	0.91	9.31	51.45
23	**Nail**	168.25	1.06	-20.38	0.92	9.17	237.94	71.58	0.92	7.54	0.80	11.89	72.51
24	**Canik 190-5**	177.87	1.23	-42.62	0.93	9.18	266.79	70.45	0.99	1.34	0.81	12.66	79.59
25	**Xanthi 81**	159.69	1.38	-87.46	0.91	13.96	497.15	77.67	0.98	8.84	0.97	4.15	10.39
	Mean	178.72						69.91					
	Confidence Interval	±5.41	±0.11					±3.65	±0.08				

The genotypes moderately adaptable to all environments were ERB-11, ERB-26 and ERB-35 lines; thus, these lines were considered as stable genotypes. The yields of ERB-11 and ERB-26 lines were lower than the average yield but remained within the confidence limits (175.80 and 177.30 kg da^{-1}). The ERB-11 and ERB-26 lines had positive regression constants (14.63 and 15.25), high coefficient of determination (0.99 and 0.94) and low coefficient of variation (1.35 and 6.68) values. The deviation from near-zero regression was recorded for the ERB-11 line (5.65). The ERB-35 line had high yield (183.11 kg da^{-1}) and a low coefficient of variation (12.13) value, while the regression constant was negative (-15.68), the coefficient of determination (0.87) was low and the deviation from the regression was high (492.99). The variability in yield can be mostly attributed to the differences in the environmental conditions of the experimental fields (Sadeghi et al. 2011). The ERB-11 and ERB-30 lines met all the conditions of the stability parameters used in the study in terms of yield values. The ERB-7, ERB-9, ERB-14, ERB-18 and ERB-21 lines also did not meet the requirements in terms of a single parameter (Table 4; Figure 2).

3.2. Quality Grade Index

The results for the variance analysis of the quality grade index were given in Table 3 and the results of the stability parameters related to yield were presented in Table 4. The graph of the adaptation classes determined using the regression coefficients and the mean yield values were shown in Figure 3. The mean quality grade index of the experiment was 69.91%. Since high-quality grade index is a desirable property, genotypes with performance higher than the overall mean value have been considered to provide the first condition of the stability. The Xanthi 81 genotype, which had a quality grade index value (77.67%) higher than the mean quality grade index value and a b_i value of 0.98, was the only genotype placed in a class suitable to all environments. The Xanthi 81 line had a positive regression constant (8.84), high r^2 value (0.97), low CV (4.15) and low S^2d (10.39) value; therefore, considered as a stable genotype. The ERB-6, ERB-14, Nail and Canik 190-5 genotypes were defined as stable genotypes since they are moderately suitable to all environments. Regression coefficients and mean yield values of these genotypes were within the confidence intervals. The ERB-6 line is remarkable with a close to zero but negative regression constant (-1.13) as well as a high coefficient of determination (0.94) and low CV (6.37) and S^2d (21.21) values. The ERB-14 line in this group stands out with a positive regression constant (1.29) and a low CV (13.60) despite the highest S^2d (84.18) and the lowest r^2 (0.79) values (Table 4; Figure 3).

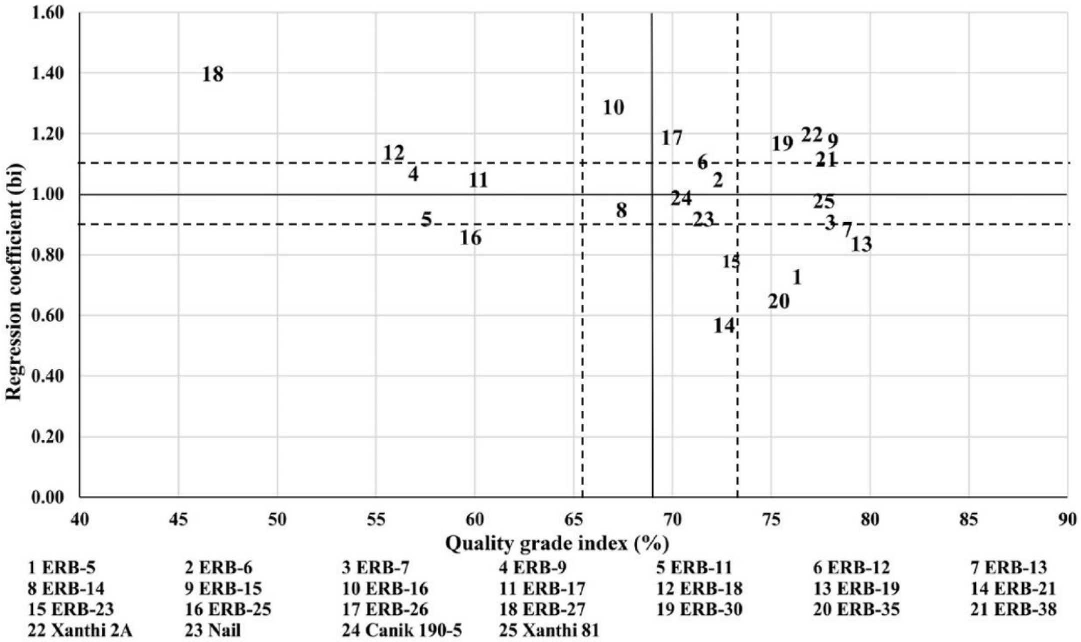

Figure 3- Stability conditions of different tobacco genotypes according to quality grade index and the regression coefficient

The quality grade index corresponds to the quantitative equivalent of quality; therefore, the value of a crop is estimated accordingly. The decline in quality is sometimes neglected under reduced production conditions; however, the quality will always be important in oriental tobacco production. Therefore, the adaptability and stability of the genotypes to different districts in terms of quality grade index are very important for tobacco breeders. In this study, Xanthi 81 was the only genotype that corresponded to all the conditions of the stability in terms of quality grade index values; thus, it was placed in the "good adaptation to all environments" class. The ERB-6 and ERB-7 lines did not meet the conditions in terms of a single parameter but attracted the attention (Table 4).

3.3. Nicotine

The results of the variance analysis for nicotine content were given in Table 3 and the related stability parameters were presented in Table 5. Adaptation classes determined using the regression coefficients and mean yield were shown in Figure 4. The low nicotine content is considered as a quality indicator for oriental tobacco. The nicotine content of Basma type tobacco demanded by the tobacco industry is between 2.00 and 2.75% (Yilmaz & Kinay 2011). The nicotine content of tobacco can be increased by cultural measures such as nitrogenous fertilization (Kinay 2010), wide planting distances (Bilalis et al. 2015) and topping (Camas et al. 2009) to meet the market demand. Therefore, the genotypes having nicotine performance over the mean value (>1.09%) have been accepted to meet the first condition of the stability (Table 5). The ERB-15 line with a nicotine content (1.19%) over the mean value and a b_i value of 1.12 was placed in the "good adaptation for all environments" class. The ERB-15 line can be considered as a stable genotype with a high r^2 (0.99), low CV (7.31) and low S^2d (0.007) values when negative regression constant (-0.037) is ignored.

The ERB-7, ERB-18 and ERB-19 lines were moderately adaptable to all environments, therefore, accepted as stable genotypes. Regression coefficients and mean nicotine contents of these genotypes were within the confidence intervals. The ERB-19 genotype in this class had the highest coefficient of determination, and the lowest CV (7.81%) and deviation from regression (S^2d; 0.007) values in this class, despite its negative regression constant (a; -0.049). The ERB-18 line was the only genotype that met all the conditions of stability parameters calculated with the nicotine values of 25 genotypes investigated. The ERB-15 and ERB-19 lines met all conditions except one parameter (Table 5; Figure 4).

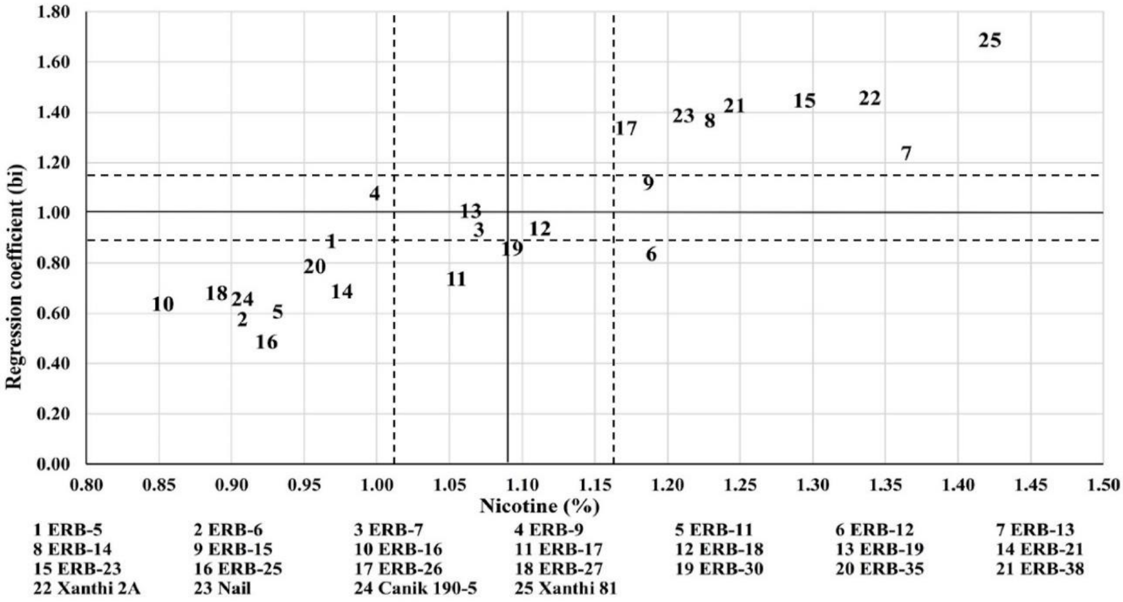

Figure 4- Stability conditions of different tobacco genotypes according to nicotine and the regression coefficient

Table 5- Values of the stability parameters for Nicotine and reducing sugar

No	*Genotypes*	*Nicotine (%)*						*Reducing sugar (%)*					
		X_{mean}	b_i	a	r^2	CV	S^2d	X_{mean}	b_i	a	r^2	CV	S^2d
1	**ERB-5**	0.97	0.89	-0.004	0.93	20.54	0.040	7.15	1.11	-2.37	0.81	13.78	0.97
2	**ERB-6**	0.91	0.60	0.256	0.84	23.91	0.047	9.27	0.65	3.67	0.75	7.45	0.48
3	**ERB-7**	1.07	0.97	0.005	0.91	23.87	0.065	9.16	0.85	1.87	0.96	2.65	0.06
4	**ERB-9**	1.00	1.08	-0.186	0.99	4.47	0.002	8.36	0.42	4.80	0.95	2.05	0.03
5	**ERB-11**	0.93	0.61	0.265	0.96	10.87	0.010	7.93	0.76	0.41	0.98	0.82	0.05
6	**ERB-12**	1.19	0.84	0.270	0.90	19.73	0.055	7.85	1.44	-4.49	0.87	12.81	1.01
7	**ERB-13**	1.36	1.24	-0.001	0.96	15.28	0.043	7.99	1.11	-1.51	0.94	6.35	0.26
8	**ERB-14**	1.23	1.37	-0.271	0.96	18.22	0.050	8.26	1.23	-2.32	0.96	5.64	0.21
9	**ERB-15**	1.19	1.12	-0.037	0.99	7.31	0.007	8.78	0.65	3.18	0.84	5.90	0.27
10	**ERB-16**	0.85	0.64	0.158	0.94	15.70	0.018	6.60	1.43	-5.70	0.93	11.00	0.53
11	**ERB-17**	1.06	0.74	0.246	0.90	19.51	0.042	8.99	0.81	2.05	0.99	2.02	0.03
12	**ERB-18**	1.11	0.94	0.078	0.95	17.03	0.036	7.94	0.93	-0.04	0.93	5.87	0.22
13	**ERB-19**	1.06	1.01	-0.049	0.99	7.81	0.007	8.92	0.57	4.01	0.97	2.05	0.03
14	**ERB-21**	0.98	0.69	0.219	0.90	19.98	0.038	10.12	1.85	-5.79	0.79	17.28	3.06
15	**ERB-23**	1.29	1.45	-0.297	0.99	3.92	0.002	8.16	0.75	1.69	0.87	6.56	0.29
16	**ERB-25**	0.92	0.49	0.391	0.85	18.11	0.028	8.15	0.69	2.18	0.88	5.75	0.22
17	**ERB-26**	1.17	1.34	-0.302	0.98	13.21	0.024	9.35	0.99	0.84	0.99	2.82	0.07
18	**ERB-27**	0.89	0.67	0.160	0.99	4.48	0.002	8.03	0.22	6.13	0.84	2.19	0.03
19	**ERB-30**	1.09	0.86	0.152	0.97	11.03	0.014	10.61	1.93	-5.96	0.95	7.24	0.59
20	**ERB-35**	0.96	0.79	0.087	0.90	7.03	0.004	7.39	0.72	1.17	0.68	12.15	0.81
21	**ERB-38**	1.25	1.43	-0.318	0.99	7.61	0.009	8.08	0.65	2.47	0.78	2.99	0.06
22	**Xanthi 2A**	1.34	1.46	-0.259	0.98	11.79	0.025	7.96	0.57	3.09	0.91	4.12	0.19
23	**Nail**	1.21	1.39	-0.322	0.99	9.87	0.014	10.57	2.35	-9.56	0.90	13.72	2.10
24	**Canik 190-5**	0.90	0.66	0.176	0.94	14.35	0.017	9.92	2.22	-9.13	0.88	14.68	2.12
25	**Xanthi 81**	1.42	1.69	-0.435	0.95	23.08	0.108	8.84	1.43	-3.39	0.83	13.06	1.33
	Mean	1.09						8.57					
	Confidence Interval	±0.07	±0.13					±0.42	±0.23				

3.4. Reducing Sugars

The results of the variance analysis for the reducing sugars were given in Table 3, and the stability parameters related to reducing sugars were presented in Table 5. The adaptation classes determined using regression coefficients and the mean reducing sugars values were shown in Figure 5. The reducing sugar ratio required by the tobacco industry for Basma type tobacco ranges between 8.00 and 13.00% (Yilmaz & Kinay 2011). Therefore, the genotypes which had reducing sugar performance higher than the mean value (>8.57%) considered meeting the first condition for stability. The ERB-7 and ERB-26 lines which had a reducing sugar value (8.99%) higher than the mean reducing sugar content and a b_i value above 1.23 were included in a "good adaptation for all environments" class. Although the regression coefficient of ERB-7 and ERB-26 lines was above the confidence intervals, they can be considered as stable genotypes due to the positive regression constants, high r^2 (0.96-0.99), low CV (2.65-2.82) and S^2d (0.06-0.07) values. The ERB-14 and ERB-17 lines, which were moderately adaptable to all environments, were stable genotypes only when evaluated in this aspect. The regression coefficients and mean reducing sugar values of these two lines were within the confidence intervals. The ERB-17 line in this group was the most stable genotype due to the positive regression constant, high coefficient of determination (r^2; 0.99), low CV (2.02%) and deviation from regression (S^2d; 0.03) values. The ERB-14 line is another genotype within the "moderately adaptable to all environments" class. Despite the negative regression constant (a; -2.32), the ERB-14 which had a high coefficient of determination (r^2; 0.96), low coefficient of variation (CV; 5.64) and low deviation from the regression (S^2d; 0.21) values, met all the conditions of the stability. The ERB-7, ERB-17 and ERB-26 genotypes met all conditions of stability parameters calculated by reducing sugar ratios of 25 genotypes. The ERB-14 line also met the conditions for all parameters except the negative regression constant (Table 5; Figure 5).

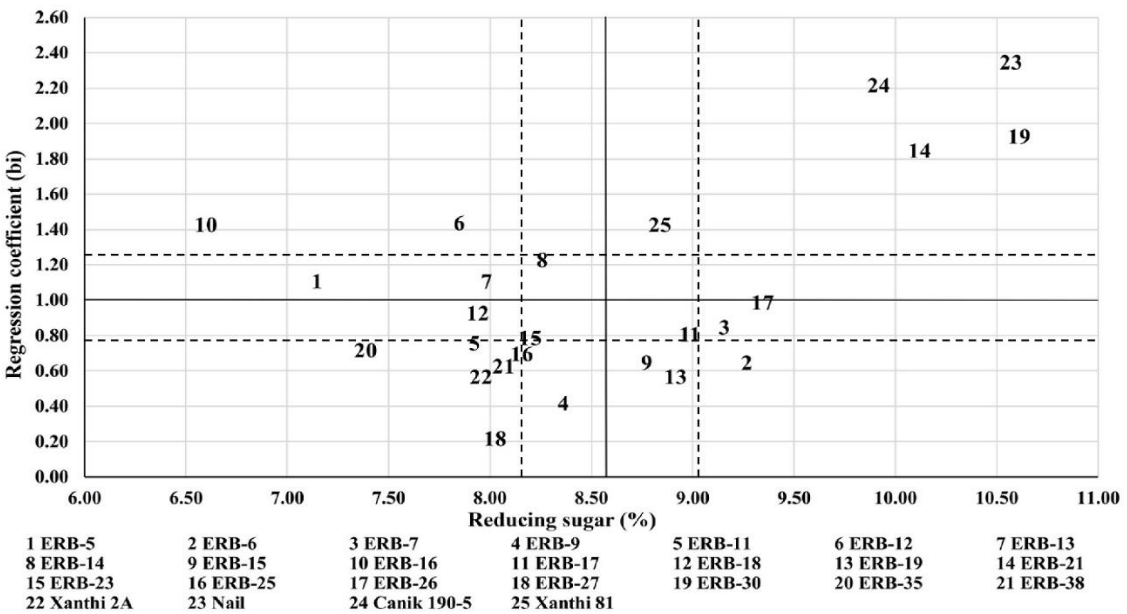

Figure 5- Stability conditions of different tobacco genotypes according to reducing sugar and the regression coefficient

4. Conclusions

The ERB-11 and ERB-30 lines met all the conditions of the stability parameters used in the study in terms of yield values. In addition, the ERB-7, ERB-9, ERB-14, ERB-16, ERB-18 and ERB-21 lines did not meet the requirements in terms of a single parameter. The ERB-6 and ERB-7 lines did not meet the stability conditions in terms of a single parameter but attracted the attention along with ERB-12, ERB-13 and ERB-38 lines in terms of quality grade index. The ERB-18 line was the only genotype that met all the conditions of stability parameters calculated by the nicotine values. The ERB-15 and ERB-19 lines met all conditions except one parameter. The ERB-7, ERB-17 and ERB-26 genotypes met all conditions of stability parameters calculated by reducing sugar. The stability results indicated that ERB-6, ERB-7, ERB-11, ERB-13, ERB-16, ERB-18, ERB-21 and ERB-30 lines are slightly affected by the environmental conditions. The results revealed that the development of (hopeful) tobacco candidates to meet the needs of producers and the sector could be continued with the aforementioned eight lines.

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