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Experimental strategies in performing value for cultivation and use experiments for the tobacco crop II: dimension of the experimental network

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ABSTRACT. In this study, we aimed to establish strategies for value for cultivation and use (VCU) experiments for the tobacco crop in the southern region of Brazil with respect to the number of environments used to assess tobacco lines. Trials of the Virginia (18 sites) and Burley (17 sites) varietal groups were conducted in the three states of the southern region of Brazil in the 2009-2010 crop season. The experiment was conducted in a completely randomized block design with four replications of 10 tobacco lines in the final stage of evaluation; the plots had 6 rows of 7 plants each, or 42 plants per plot. The cured leaf weight per hectare (kg/ha) was obtained. To evaluate stability, the ecovalence and additive main effects and multiplicative interaction models were adopted. In addition, joint analyses of variance were carried out considering different site numbers by simulating resampling. The site number ranged from 2 to 17 or 2 to 16, depending on the varietal group, and sites were selected at random without replacement. The process was repeated 2000 times for each number of sites. All analyses were performed using the R software. The results are very similar for both varietal groups. There is no advantage of using a large number of sites for VCU experiments in the southern region of Brazil because many sites contributed little to the interaction or did not discriminate the tobacco lines. Furthermore, the classification of the best lines is very similar to that obtained in the total number of evaluated sites.

Key words: Experimental design; Genotype by environment interaction; Monte Carlo simulation; Plant breeding

INTRODUCTION

As required by the Ministry of Agriculture in Brazil, value for cultivation and use (VCU) experiments, must be carried out to register new cultivars. By law, to commercialize seeds and propagules of any cultivar, whether protected or not, registration is indispensable (Brasil, 1997). Thus, according to the crop species, the setup of the standards for performing such trials varies, and they are defined by the Ministry and follow the opinion of specialists in statistics experimentation with the crop.

The final evaluation stage of the lines aiming at their approval as cultivars for cultivation is costly and demands a large amount time from breeders, resulting in the need for the most precise trials possible. However, the main difficulty of these trials is the occurrence of interaction between lines and locations (environments). Because of this interaction, the lines behave differently in the different sites. This is a known fact for most crops (Pacheco et al., 2003; Terasawa Jr. et al., 2008; Bertoldo et al., 2009; Pereira et al., 2009). The tobacco crop is no different.

To lessen the effect of this interaction, the VCU experiments should be conducted in great number of environments, seeking to reduce errors in identification of the best lines that may be recommended for the region for which the new cultivars are intended. The question, however, is what is the best number of sites. Obviously, this number varies according to the crop and the environmental diversity of the region. This diversity contributes to variation in the importance of the line with environment interaction.

Knowing this number for each crop and/or region is highly desirable because if few sites are used, the precision of the recommendation will be questioned. If an excessive number of sites is used, the efficiency of the breeding program will diminish because the cost of each trial is normally high. With other crops grown in Brazil, numerous studies expressed a view toward ecological zoning (Pacheco et al., 2003; Terasawa Jr. et al., 2008; Bertoldo et al., 2009; Pereira et al., 2009). These studies, however, have a different focus from that presented here. Because information for tobacco growing was not found in the literature, this study was performed to present strategies for carrying out experiments of the VCU network for the tobacco crop in the southern region of Brazil with respect to the dimension of the trial network for the Burley and Virginia varietal groups.

MATERIAL AND METHODS

The trials of the tobacco line evaluation network of the Virginia varietal group (18 sites) and Burley varietal group (17 sites) were analyzed. The site list of these trials conducted in the three states of the southern region of Brazil in the 2009-2010 crop season is presented in Table 1. The experiment was conducted in a completely randomized block design with four replications consisting of 10 tobacco lines in the final stage of evaluation. The plots had 6 rows of 7 plants each, or 42 plants per plot.

Table 1. List of sites in which the value for cultivation and use (VCU) trials were conducted during the 2009-2010 season for the varietal groups Virginia and Burley in southern Brazil.

Virginia			Burley		
ID	Site	State	ID	Site	State
1	Vale do Sol	RS	1	Coronel Freitas	SC
2	Venâncio Aires	RS	-	-	-
3	Boqueirão do Leão	RS	-	-	-
4	Fontoura Xavier	RS	4	Iraí	RS
5	São Lourenço do Sul	RS	5	Seberi	RS
6	Pelotas	RS	6	Cunha Porã	SC
7	Arroio do Padre	RS	7	Marmeleiro	PR
-	-	-	8	Marechal Cândido Rondon	PR
-	-	-	9	Marechal Cândido Rondon	PR
10	Ímbuia	SC	10	Missal	PR
11	Agronômica	SC	11	Marechal Cândido Rondon	PR
12	Taió	SC	-	-	-
13	Rio Negro	PR	13	Toropi	RS
14	Rio Negro	PR	-	-	-
15	Rio Negro	PR	15	Alegre do Marco	SC
16	Rio Negro	PR	16	Abelardo Luz	SC
17	Rio Negro	PR	17	Abelardo Luz	SC
18	Rio Negro	PR	18	Campo do Tenente	PR
-	-	-	19	Campo do Tenente	PR
20	Campo do Tenente	PR	20	Rio Negro	PR
21	Rio Negro	PR	-	-	-
-	-	-	22	Rio Negro	PR

The cured leaf weight per hectare (kg/ha) was obtained, and analyses of variance were performed for each location. Then, after verifying the homogeneity of the variances, joint analysis of variance was carried out. The model that was adopted was similar to that presented by Ramalho et al. (2012): $y_{ij(k)} = \mu + a_j + r_{k(j)} + g_i + (ga)_{ij} + e_{ik(j)}$, where $y_{ij(k)}$ is the observation in replication k within location j of line i; μ is the constant inherent to all plots, which, by the restriction imposed, is the general mean of the observations; a_j is the effect of site j with j = 1, 2, 3, 4, … , 18 or 17 for the tobacco varietal group (Burley or Virginia, respectively); $r_{k(j)}$ is the effect of replication k within site j with k = 1, 2, 3, or 4; g_i is the effect of line i (i = 1, 2, … , 10); $(ga)_{ij}$ is the interaction of line i with site j; and $e_{ik(j)}$ is the error term with $e \sim N(0, \sigma^2)$.

After that, sources of variation in lines within environments ($g_{i(j)}$) were divided into differences among lines (g_i) and the line by site interaction [$(ga)_{ij}$].

The interaction between lines and sites was also studied using the additive main effects and multiplicative interaction (AMMI) method according to the following model (Duarte and Vencovsky, 2001): $y_{ij} = \mu + a_j + g_i + \Sigma\, \lambda_k \gamma_{ik} \alpha_{jk} + \rho_{ij} + \varepsilon_{ij}$, where y_{ij} is the observed mean of genotype i in site j; μ is the general mean; a_j is the effect of the environment (site j) and j = 1, 2, … , 17 or 18 according to the tobacco varietal group (Burley or Virginia, respectively); g_i is the effect of genotype i with i = 1, 2, ... , 10; λ_k is the eigenvalue of the c^{th} principal component related to the genotype by environment interaction; γ_{ik} is the eigenvector of the c^{th} principal component related to the genotype i; α_{jk} is the eigenvector of the c^{th} principal component related to the site j; ρ_{ij} is the residue or noise that is not explained by the principal components; and ε_{ij} is the error term, with $e \sim N(0, \sigma^2)$. The fitted model follows the F-Snedecor test of Gollob (1968), and the results were used in the biplot graph representation (Gabriel, 1971).

To evaluate the stability of the trial locations, a partition of the sum of squares of the line by site interaction, named ecovalence (ω_j) (Wricke and Weber, 1986), was used because of the easy interpretation and complementarity to the AMMI analysis. This partition is obtained by $\omega_j = \sum[(ga)_{ij}]^2$, in which ω_j is the ecovalence of site j and $(ga)_{ij}$ is the estimate of the effect of line i by site j interaction. Through the principle of the method, the sum of squares of the effects $[(ga)_i]^2$ recovers the sum of squares of the lines by environment interaction; thus, the relative percentage of the sum of squares of the interaction due to each location [ω_j(%)] was estimated.

In addition, joint analyses of variance were carried out considering a different number of sites. The resampling method was used in the following manner: an algorithm over the data spreadsheet sampled p evaluation sites without replacement, performed analysis of variance. The following estimates were stored: mean square of the interaction (V_i), mean square of the line (V_t), and the three performance best lines in the mean of the p locations.

The number of situations for each site sample size is a function of the combination of the total site number and the site sample size ($n!/[(n - p)!\ p!]$), in which n is the total number of trials and p is the trial sample size. For p in which the number of situations is less than 2000, all the respective analyses were carried out. In the other cases, the algorithm performed 2000 cases. The number of sites (p) ranged from 2 to 17 or 16, depending on the varietal group.

All the analyses were carried out and/or implemented using the R software (R Development Core Team, 2011).

RESULTS AND DISCUSSION

Initially, we will comment on the results in reference to the Burley varietal group (Table 2). A significant difference (P < 0.01) was detected for the local, replications within sites, lines, and the line by site interaction variation sources. The occurrence of variation among sites was expected in view of the fact that environments differing in soil fertility, climatic conditions, and crop management were involved because the trials were conducted on farm properties. The replications within trial sources of variation show that local control of variation by block randomization was effective and that, in general, the performance of the replications differs within sites.

The occurrence of variation among lines and among sites is important so that the occurrence of line by site interaction can be detected if it occurs. The sum of squares of the line by site interaction was 2.1 times greater than the sum of squares of lines, showing the importance of the interaction in the tobacco crop for that region. The occurrence of this interaction has frequently been reported in other crops in the same region (Terasawa Jr. et al., 2008; Bertoldo et al. 2009; Pereira et al. 2009). The first proof of the existence of the interaction may be observed as the source of variation among lines within each site. In some sites, like sites 1, 4, 12, 14, 16, 18, and 20, a significant difference among the lines was not detected. In other words, it was not possible to discriminate the lines in these sites. However, in the other 10 sites, the lines could be discriminated (Table 2).

Seeking to better elucidate the occurrence of the line by site interaction, all analyses of variance were performed for environments two by two (Tables 3 and 4). There was wide varia-

Table 2. Summary of joint analysis of variance of evaluation trials of the tobacco inbred varietal groups Virginia and Burley for cured leaf weight per hectare (kg/ha) in southern Brazil during the 2009-2010 season.

Source	Virginia			Burley		
	d.f.	Mean square	P	d.f.	Mean square	P
Sites	17	15,194,817	<0.001	16	14,020,781	<0.001
Block/Site	54	337,225	<0.001	51	136,815	<0.001
Inbreds	9	1,249,231	<0.001	9	768,121	<0.001
Inbreds x Sites	153	189,569	<0.001	144	97,376	<0.001
Inbreds/Site 1	9	20,495	0.065	9	38,914	0.628
Inbreds/Site 2	9	166,777	0.157	-	-	-
Inbreds/Site 3	9	86,962	0.649	9	-	-
Inbreds/Site 4	9	362,935	<0.001	9	50,950	0.410
Inbreds/Site 5	9	221,640	0.043	9	231,650	<0.001
Inbreds/Site 6	9	98,797	0.553	9	107,094	<0.001
Inbreds/Site 7	9	128,628	0.339	9	299,422	<0.001
Inbreds/Site 8	-	-	-	9	119,882	<0.001
Inbreds/Site 9	-	-	-	9	205,299	<0.001
Inbreds/Site 10	9	222,774	0.042	9	90,675	0.060
Inbreds/Site 11	9	254,582	0.019	-	185,900	<0.001
Inbreds/Site 12	9	248,915	0.022	9	-	-
Inbreds/Site 13	9	71,967	0.769	-	38,945	0.620
Inbreds/Site 14	9	47,192	0.927	9	-	-
Inbreds/Site 15	9	60,566	0.851	9	111,634	0.018
Inbreds/Site 16	9	383,983	<0.001	9	82,724	0.093
Inbreds/Site 17	9	420,811	<0.001	9	144,342	0.002
Inbreds/Site 18	9	182,592	0.111	9	80,891	0.102
Inbreds/Site 19	-	-	-	9	168,679	<0.001
Inbreds/Site 20	9	310,016	0.004	-	70,585	0.173
Inbreds/Site 21	9	997,825	<0.001	9	-	-
Inbreds/Site 22	-	-	-	-	298,553	<0.001
Residual	485	113,722	-	455	49,418	-

tion in the estimates of the mean squares of the line by site interaction. The contribution of each site to the interaction was different, as was expected. If the number of pairs of sites in which the interaction was not significant is considered for a fixed site, the interaction was not significant in site 7 in two pairs and in site 22 in one pair. However, in sites 6 and 15, there were 13 and 12 pairs of sites in which the interaction was not significant (P < 0.05), respectively.

Confirming what was stated above, sites 7 and 22 contributed the most to the interaction according to the ecovalence method (Wricke and Weber, 1986). Sites 6, 10, 13, 16, and 17 were among those that least contributed to the interaction (Tables 5 and 6).

The AMMI model was applied to study stability, presupposing that the effects of environments and lines are additive and that the interaction is multiplicative (Gauch Junior and Zobel, 1996). It may be observed that the first three principal components were significant (P < 0.05) and explained 71% of the variation. Three components are difficult to graphically represent and to explain the contribution of the sites to the interaction. For that reason, we used the first two components, which were significant (P < 0.01) and explained 58.7% of the variation (Table 7).

In graphic analysis (Figure 1), the greater the number of sites that are placed near the origin, the less they contributed to the interaction (Duarte and Vencovsky, 2001). If sites 6, 10, 15, and 16 are considered, the coincidence between the number of pairs in which each site participated and the pairs that were not significantly different was very high. Using the ecovalence model, for example, sites 7 and 22 were those with the greatest number of pairs with significant interactions; these sights are the most distant from the origin. A relevant observation is that even with the two principal components explaining only 58.7% of the variation, the agreement with the other strategies that were used was very high.

Table 3. Matrix decomposition of the sites by inbred interaction (two by two) for site evaluation trials of the tobacco inbred varietal group Virginia according to the cured leaf weight per hectare (kg/ha).

Sites	Sites																	
	1	2	3	4	5	6	7	10	11	12	13	14	15	16	17	18	20	21
1		28,607.7	126,042.0	323,010.5	222,649.3	161,708.1	79,779.7	57,235.7	104,923.3	90,656.1	81,965.4	135,187.1	79,286.7	177,024.2	269,919.3	166,210.4	174,967.5	700,292.1
2	0.986		107,398.3	363,667.8	202,599.3	168,721.4	62,418.2	54,090.2	80,153.1	71,246.8	43,882.4	107,892.5	69,127.2	153,058.7	222,529.1	114,649.0	92,729.3	570,419.9
3	0.355	0.486		335,917.0	179,085.5	34,012.5	54,182.5	113,208.6	108,356.1	111,794.2	51,856.8	61,762.8	62,548.4	161,323.7	197,969.3	97,078.2	148,238.3	476,390.5
4	0.003	0.001	0.002		216,562.2	289,932.3	342,189.0	320,125.5	491,466.3	546,116.5	283,217.3	181,856.6	219,869.8	446,092.8	414,602.2	297,699.3	488,024.9	654,650.7
5	0.042	0.069	0.12	0.049		161,455.6	143,735.0	133,529.7	237,106.0	315,232.2	137,573.7	95,928.4	70,052.4	113,170.9	138,318.9	97,907.6	171,780.0	360,350.2
6	0.176	0.151	0.975	0.007	0.176		99,900.8	172,666.4	130,140.5	152,409.1	103,649.9	60,679.0	78,644.3	238,132.8	301,373.1	172,387.7	246,702.7	598,161.5
7	0.708	0.839	0.891	0.002	0.254	0.544		40,872.6	114,697.2	109,262.3	63,681.8	102,559.7	54,540.0	88,535.5	144,899.7	91,932.3	129,185.5	545,025.8
10	0.872	0.891	0.443	0.003	0.309	0.138	0.954		155,672.1	170,732.1	73,021.8	120,453.7	68,162.4	49,301.9	136,980.3	70,410.3	110,320.7	475,626.9
11	0.505	0.705	0.479	<0.001	0.029	0.330	0.432	0.199		101,720.0	132,967.5	161,903.7	123,183.2	220,809.9	278,358.2	199,503.9	130,330.1	638,395.9
12	0.619	0.775	0.453	<0.001	0.004	0.213	0.472	0.144	0.530		92,916.5	178,071.8	128,035.8	265,594.2	359,278.7	223,435.0	183,242.7	788,359.1
13	0.690	0.942	0.904	0.009	0.286	0.515	0.830	0.761	0.313	0.601		33,364.6	25,841.8	135,218.4	171,359.4	61,961.5	95,046.8	410,012.3
14	0.300	0.482	0.843	0.113	0.576	0.850	0.523	0.392	0.175	0.123	0.976		27,472.4	189,272.0	215,225.7	95,516.0	169,591.9	413,407.3
15	0.712	0.791	0.838	0.045	0.784	0.717	0.888	0.798	0.374	0.342	0.991	0.988		112,324.9	138,163.8	81,074.8	130,100.6	433,704.1
16	0.126	0.210	0.177	<0.001	0.443	0.029	0.636	0.917	0.044	0.014	0.300	0.095	0.449		59,246.6	65,349.3	96,550.8	330,737.5
17	0.012	0.042	0.077	<0.001	0.282	0.005	0.248	0.290	0.010	0.001	0.142	0.051	0.283	0.860		85,806.2	120,615.2	217,855.3
18	0.159	0.432	0.567	0.006	0.560	0.139	0.609	0.781	0.075	0.042	0.842	0.580	0.697	0.818	0.659		48,921.0	239,656.3
20	0.132	0.602	0.232	<0.001	0.141	0.023	0.335	0.464	0.328	0.109	0.583	0.148	0.330	0.571	0.391	0.919		288,789.8
21	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	<0.001	0.002	0.048	0.028	0.007	

Trials were in southern Brazil during the 2009-2010 season. Values above the diagonal are the mean squares and those below the diagonal are the significance of the respective term.

Table 4. Matrix decomposition of the sites by inbred interaction (two by two) for site evaluation trials of the tobacco inbred varietal group Burley according to the cured leaf weight per hectare (kg/ha).

Sites	Sites																
	1	4	5	6	7	8	9	10	11	13	15	16	17	18	19	20	22
1		36,048.0	166,587.0	92,502.3	189,387.6	92,286.3	105,867.6	77,540.8	146,181.6	47,529.4	105,931.3	79,740.2	105,942.9	80,077.4	125,448.5	56,616.8	231,252.9
4	0.682		161,208.1	70,829.8	172,931.2	97,933.5	98,986.3	81,082.2	122,902.3	47,857.2	79,462.6	72,815.2	75,040.8	33,719.1	97,413.7	38,513.9	174,572.6
5	<0.001	0.001		86,919.6	28,654.1	116,625.3	235,050.3	81,619.5	192,191.6	134,975.7	96,164.0	67,022.5	149,879.5	100,646.9	178,271.4	177,369.0	82,569.4
6	0.054	0.171	0.074		111,267.6	50,469.2	88,046.0	42,840.9	60,672.8	34,332.9	46,600.8	25,059.2	43,789.3	56,427.9	137,445.7	67,926.9	129,716.9
7	<0.001	<0.001	0.814	0.018		165,169.0	259,975.8	107,775.4	269,461.9	171,248.5	101,806.3	104,277.9	180,978.1	92,048.0	226,006.2	242,213.4	119,637.8
8	0.055	0.040	0.013	0.422	0.001		65,892.3	11,639.7	80,131.4	25,766.2	65,735.6	32,178.8	41,732.0	116,393.0	79,893.0	56,966.1	152,236.2
9	0.025	0.037	<0.001	0.069	<0.001	0.217		91,747.3	85,803.7	51,383.3	98,730.7	61,400.5	28,615.0	164,090.8	78,295.9	88,011.3	263,691.8
10	0.122	0.101	0.098	0.555	0.022	0.989	0.056		90,565.1	27,528.5	38,013.7	29,891.1	43,791.1	72,027.0	85,350.4	74,782.2	105,101.3
11	0.002	0.009	<0.001	0.276	<0.001	0.106	0.078	0.060		46,807.5	88,750.8	61,235.0	39,058.0	138,965.2	105,533.8	86,093.9	150,789.0
13	0.471	0.466	0.004	0.714	<0.001	0.859	0.407	0.832	0.484		42,495.2	25,324.5	29,130.7	67,106.2	61,963.4	34,921.8	142,816.8
15	0.025	0.110	0.044	0.487	0.032	0.219	0.038	0.645	0.067	0.561		28,399.4	37,945.6	41,294.7	92,717.8	120,785.6	121,287.4
16	0.109	0.155	0.206	0.870	0.027	0.753	0.267	0.793	0.269	0.866	0.818		28,654.6	69,590.1	67,967.5	69,765.5	121,479.0
17	0.025	0.138	0.002	0.538	<0.001	0.575	0.815	0.538	0.625	0.806	0.646	0.814		92,529.0	62,854.1	79,129.0	147,488.3
18	0.107	0.725	0.034	0.331	0.056	0.013	0.001	0.161	0.003	0.205	0.584	0.182	0.054		137,216.3	98,632.0	113,832.5
19	0.008	0.041	<0.001	0.004	<0.001	0.108	0.117	0.080	0.026	0.260	0.054	0.197	0.250	0.004		93,510.5	138,154.9
20	0.329	0.636	<0.001	0.197	<0.001	0.324	0.070	0.140	0.077	0.703	0.010	0.180	0.112	0.038	0.051		193,445.5
22	<0.001	<0.001	0.094	0.006	0.011	0.001	<0.001	0.026	0.002	0.002	0.010	0.010	0.002	0.015	0.003	<0.001	

Trials were in southern Brazil during the 2009-2010 season. Values above the diagonal are the mean squares and those below the diagonal are the significance of the respective term.

Table 5. Matrix decomposition of the sites by inbred interaction for sites following the Wircke and Weber (1986) and sum of the ecovalence (ω_i) for the Virginia varietal group according to the cured leaf weight per hectare (kg/ha).

Sites	Inbreds										ω_i	ω_i (%)
	1	2	3	4	5	6	7	8	9	10		
1	12,870.2	5280.8	198,468.2	179,844.7	605,071.5	113,402.1	68,233.6	49,851.9	69,785.3	44,005.7	1,346,814.0	4.66
2	95,174.2	3506.9	158,760.6	34,236.5	160,330.9	265,021.4	91,606.5	8231.1	54,624.6	6126.9	877,619.6	3.03
3	126,093.7	6133.9	106,176.7	725.3	76,998.8	48,663.3	33,282.3	193,710.4	77,183.4	158,702.3	827,670.1	2.86
4	79,156.0	358.4	443,686.1	6590.4	165,863.5	1,324,450.4	226,275.7	1,755,955.1	40,428.7	596,563.8	4,639,328.0	16.04
5	71,287.3	172,873.9	78,990.4	286,727.0	2765.4	52,074.2	13,302.0	231,625.2	241,357.0	241,302.4	1,392,304.8	4.81
6	586,882.7	61,849.3	379,285.7	101,477.2	14,280.4	94,625.0	40,034.8	135,526.7	48,975.9	34,544.1	1,497,481.8	5.18
7	124,113.0	109,904.8	34,651.0	3054.7	4870.2	6740.8	294,703.1	25,736.3	1407.7	10,317.4	615,498.9	2.13
10	7543.5	152,388.0	21,816.0	1415.2	159,650.9	753.6	177,254.1	46,257.5	2638.8	108,949.8	678,667.3	2.35
11	59,144.7	52,707.4	291,867.6	8432.9	39,159.1	1,008,020.7	30,462.3	234,764.9	66,780.4	18,231.9	1,809,571.8	6.25
12	54,965.3	202,798.0	296,477.7	88,257.1	142,780.8	328,618.2	190,284.0	252,783.2	67,428.0	618,935.5	2,243,327.9	7.75
13	62,927.3	4112.8	58,224.6	3067.1	46,899.7	9711.6	2599.4	91.7	34.5	200,188.7	387,857.4	1.34
14	1564.5	16,340.8	271,959.8	82,322.5	9774.0	140,810.8	189,037.5	26,479.3	6453.1	26,316.8	771,059.0	2.67
15	35,155.0	7971.1	56,857.3	211.2	47,923.1	5140.6	15,708.7	16,711.9	106,133.3	6364.0	298,176.1	1.03
16	6.2	85,977.4	732,825.6	15,742.5	487.8	2285.1	116,735.4	95,002.9	126,936.2	87,453.5	1,263,452.6	4.37
17	132.3	210,882.1	778,016.3	115,961.4	322,722.3	1211.2	124.7	77,158.7	330,373.2	21,528.4	1,858,110.6	6.42
18	117,616.9	1171.8	279,420.4	3424.5	52,457.8	75,431.3	1233.1	10,676.0	51,878.7	1.4	593,311.8	2.05
20	358,385.7	54,957.9	285,585.9	3156.3	119,188.3	319,397.2	5229.5	7289.0	47,119.0	5871.3	1,206,180.0	4.17
21	1,464,833.9	33,276.7	2,095,986.8	11,809.0	1,127,603.1	255,022.7	1,280,370.1	131,387.8	224,279.0	360.0	6,624,929.0	22.90

Trials were in southern Brazil during the 2009-2010 season.

Table 6. Matrix decomposition of the sites by inbred interaction for sites following the Wircke and Weber (1986) and sum of the ecovalence (ω_i) for the Burley varietal group according to the cured leaf weight per hectare (kg/ha).

Sites	Inbreds										ω_i	ω_i (%)
	1	2	3	4	5	6	7	8	9	10		
1	45,849.3	148,679.2	403,793.2	60,962.0	26,356.2	97,743.9	162,318.9	37,462.3	1207.0	3189.7	987,561.7	7.03
4	3005.7	68,533.7	133,332.5	62,898.2	9340.5	303,535.0	2776.0	20,549.8	67,110.9	31,747.2	702,829.6	5.01
5	10,638.9	581.3	35,544.4	109,637.6	5955.9	94,790.3	33,623.7	106,908.5	42,544.3	937,996.1	1,378,221.0	9.82
6	32,860.5	7096.3	32,185.4	10,659.6	129,381.2	107,262.7	1278.9	2361.8	66,619.6	606.5	390,312.4	2.78
7	27,414.9	83,543.8	10,120.9	18,508.3	21,055.2	39,326.8	96,448.7	50,130.4	18,308.2	1,499,577.8	1,864,434.9	13.28
8	128,863.4	8465.9	1357.7	97,659.3	22,483.8	53,019.6	3134.7	118,231.5	31,556.8	35,674.7	500,447.4	3.56
9	54,866.0	22,440.1	3357.3	422,883.0	1959.3	61,518.8	2832.6	81,543.6	60,835.0	443,007.1	1,155,242.9	8.23
10	116,023.9	1155.4	21,131.4	50,468.6	18,115.4	38,068.9	18,731.8	13,971.4	26,404.6	18,433.5	322,504.9	2.3
11	50,681.1	261,540.8	90,332.0	10,732.0	23,822.7	90,216.1	17,001.1	84,130.1	11,393.9	434,251.4	1,074,100.9	7.65
13	6252.9	5386.0	4795.2	37,676.7	1563.9	8401.5	10,730.5	28,140.7	362.6	114,770.2	218,080.2	1.55
15	1464.9	21,094.5	99,794.6	7700.9	1575.8	2755.2	208,174.9	59,732.3	49,458.3	12,966.9	464,718.3	3.31
16	23,708.2	1364.5	94,681.0	12,222.4	56.3	3685.7	7927.8	23,042.7	17,054.3	685.0	184,427.9	1.31
17	21,616.2	104,659.1	37,115.1	42,027.0	4153.3	8881.2	83,817.1	4468.7	2.7	146,595.2	453,335.5	3.23
18	425.4	131,471.1	9889.8	179,432.9	14,243.5	56,710.7	79,869.5	145,963.7	23,795.5	82,381.9	724,183.8	5.16
19	55,519.3	79,614.6	8612.4	23,763.7	565,359.6	162,723.6	14,795.8	449.4	35,907.3	112,377.5	1,059,123.2	7.54
20	53,511.5	13,721.7	72,359.5	41,268.0	34,149.6	24,992.5	255,366.7	15,375.5	27,958.4	293,252.1	831,955.3	5.92
22	64,528.9	378,299.5	713.0	452,855.2	268,328.1	148,001.7	21,892.7	63,102.4	65.5	332,551.2	1,730,338.2	12.32

Trials were in southern Brazil during the 2009-2010 season.

Table 7. Summary of the analysis of the sites by inbred interaction according to the additive main effects and multiplicative interaction model.

Principal components	Virginia			Burley		
	% explained	d.f.	P	% explained	d.f.	P
1	42.10	25	<0.001	40.10	24	<0.001
2	25.10	23	<0.001	18.60	22	<0.001
3	11.50	21	0.110	12.30	20	0.020
4	7.50	19	0.460	9.50	18	0.080
5	4.90	17	0.770	8.60	16	0.080
6	3.30	15	0.900	4.60	14	0.530
7	2.60	13	0.920	3.20	12	0.700
8	2.00	11	0.930	2.20	10	0.800
9	1.00	9	0.980	0.90	8	0.950
10	0.00	7	1	0.00	6	1

Model is based on the cured leaf weight per hectare (kg/ha) for the Virginia and Burley varietal groups from the 2009-2010 season.

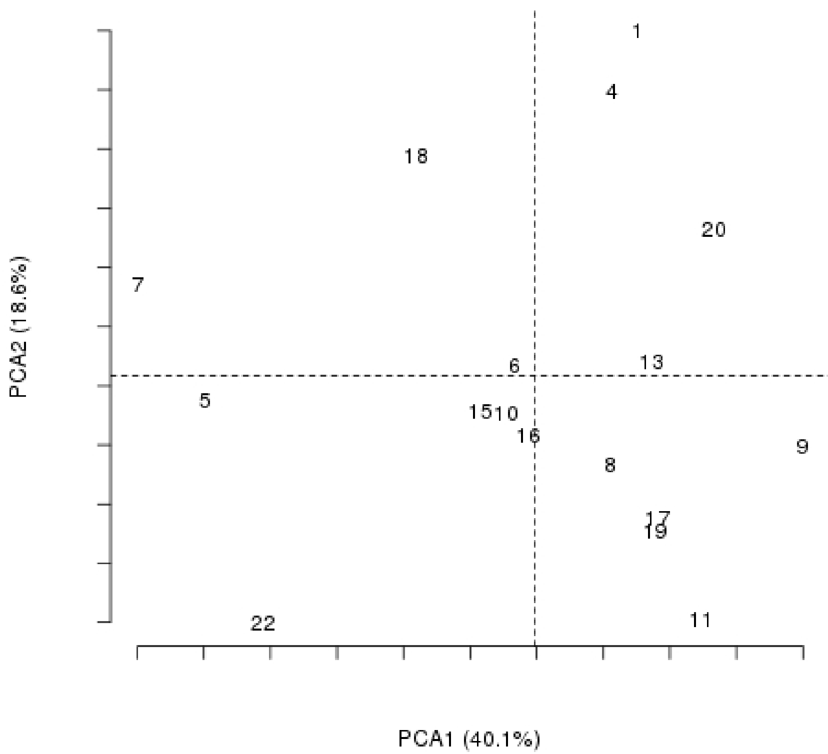

Figure 1. Biplot representation of the sites by inbred interaction analysis using the additive main effects and multiplicative interaction model. This illustrates the first versus second principal components for the cured leaf weight in the Burley varietal group during the 2009-2010 season.

In the VCU experiment, identifying the best line or the three best lines is of interest. Therefore, the validity of the best lines that were identified for different number of sites was estimated by resampling simulations (Table 8). In an average of 2000 simulations, the occurrence of the best three lines that were identified in the experiment with the 17 locations was very high. With two trial sites, the first (best) line appears among the three best lines in 76% of 136 situations; the same line was identified as the best line in 36% of cases. Using seven sites, the best line was identified among the three best lines in 100% of cases, and this pattern continued until all sites were included in the analysis.

Table 8. Percentage of coincidence in the classification of the three best lines for different numbers of sites that were evaluated for cured leaf weight per hectare (kg/ha) from the Virginia and Burley varietal groups that were grown in southern Brazil during the 2009-2010 season.

Number of sites	Virginia		Burley	
	1st among top 3	1st as 1st	1st among top 3	1st as 1st
2	0.81	0.40	0.76	0.36
3	0.80	0.43	0.87	0.43
4	0.81	0.43	0.91	0.48
5	0.74	0.44	0.96	0.52
6	0.75	0.43	0.99	0.54
7	0.78	0.45	1.00	0.57
8	0.79	0.47	1.00	0.59
9	0.80	0.48	1.00	0.62
10	0.82	0.49	1.00	0.62
11	0.82	0.52	1.00	0.63
12	0.85	0.53	1.00	0.65
13	0.87	0.56	1.00	0.67
14	0.90	0.61	1.00	0.72
15	0.96	0.60	1.00	0.76
16	0.98	0.65	1.00	0.82
17	0.99	0.73	-	-

We also observed that the estimate of the relationship between the quadratic components of the line by location interaction (V_i) and of lines (V_t), obtained from joint analysis, was very similar when different numbers of locations were used. The quadratic component of the interaction was always superior to that of the line component, once more showing the importance of interaction in the studied region (Table 9).

Table 9. Ratio between sites by inbred interaction quadratic components (V_t) and the tobacco inbred component (V_i) and the respective standard deviation ratio with varied numbers of sites.

Sites	Virginia		Burley	
	V_t / V_i	σV_t / σV_i	V_t / V_i	σV_t / σV_i
2	1.29	1.04	1.20	0.93
3	1.29	1.06	1.21	0.90
4	1.31	1.07	1.22	0.91
5	1.23	1.06	1.19	0.89
6	1.30	1.07	1.19	0.89
7	1.31	1.08	1.19	0.87
8	1.31	1.08	1.20	0.85
9	1.28	1.09	1.21	0.89
10	1.29	1.09	1.23	0.85
11	1.28	1.10	1.21	0.84
12	1.29	1.06	1.22	0.86
13	1.30	1.11	1.22	0.86
14	1.29	1.12	1.22	0.85
15	1.29	1.08	1.22	0.85
16	1.29	1.08	1.22	0.84
17	1.29	1.08	1.22	-
18	1.29	-		

Values are based on the cured leaf weight per hectare (kg/ha) in the Virginia and Burley varietal groups that were grown in southern Brazil during the 2009-2010 season.

In light of the above, it is not necessary to use 17 sites. For good security, six to eight locations would be sufficient to accurately recommend a line. A key question is which locations should be chosen. Considering the AMMI model and the analysis of variance, locations 5 (Seberi, RS), 7 (Marmeleiro, PR), 9 (Marechal Cândido Rondon, PR), 11 (Marechal Cândido Rondon, PR), 15 (Alegre do Marco, SC), 17 (Abelardo Luz, SC), 19 (Campo do Tenente, PR), and 22 (Rio Negro, PR) may be identified because they include all three states of the southern region of Brazil and would thus allow recommendations for the entire region that grows this tobacco varietal group. This choice was based on the fact that it was possible to discriminate the lines in these sites; that is, the difference among the lines is expressed and the lines are distant on the biplot graph. Sites like numbers 1, 4, 18, and 20 would be chosen because of their distance from the graph origin; however, in these locations, it was not possible to discriminate the evaluated lines (Figure 1 and Table 2).

The results for the Virginia varietal group were quite similar to those described for the Burley group. In the joint analysis of variance, the line by location interaction was significant. In this group, which included 18 sites, it was only possible to detect a significant difference among the lines in nine sites ($P < 0.05$) (Table 2).

When analysis of variance was conducted on pairs of sites, wide variation was observed in the estimates of the interaction mean square (Table 3). All interactions were significant for pairs involving site 21, whereas the interaction was significant ($P < 0.05$) in only two of the pairs involving site 13. As expected, site 21 contributed the most to the interaction by the estimate of ecovalence, and sites 13 and 15 contributed the least (Table 6).

When the AMMI model was applied to the stability experiments involving the Virginia group (Table 5), the F-test was significant ($P < 0.01$) in only the first two principal components. These two components explained 67.2% of the variation, which was very similar to the result that was obtained for the Burley varietal group. The biplot representation shows that the results coincided with those already mentioned. In other words, sites 4 and 21 were among those that most contributed to the interaction. Site 13 was situated nearest to the origin and, therefore, contributed the least to the interaction. In this case, other sites of the Virginia varietal group were also situated near the origin, including sites 3, 7, 10, 15, and 18 (Figure 2). These sites also contributed little to the interaction by the ecovalence estimate.

Through simulation, the identification coincidence of the best line was evaluated considering 2000 cases. Although the coincidence values were slightly inferior to those obtained in the Burley group, the values were high. With two environments (153 situations), the identification coincidence of the best line relative to 18 sites among the three best lines was 81%, while the best line was identified as the best line in 40% of cases. Considering seven sites, the best line was among the three best lines in 78% of the cases. It was also observed that the V_t / V_i ratio was very similar to that observed for the Burley varietal group. Here also, the V_t / V_i ratio was practically unchanged by the number of sites involved in the joint analysis. The locations for carrying out future VCU evaluations should be chosen considering the discrimination of the lines, the contribution to the interaction, and the importance of the crop in the southern region of Brazil. In practice, sites 1 (Vale do Sol, RS), 4 (Fontoura Xavier, RS), 5 (São Lourenço do Sul, RS), 11 (Agronômica, SC), 12 (Taió, SC), 16 (Rio Negro, PR), 17 (Rio Negro, PR), 20 (Campo do Tenente, PR), and 21 (Rio Negro, PR) should be chosen for analysis.

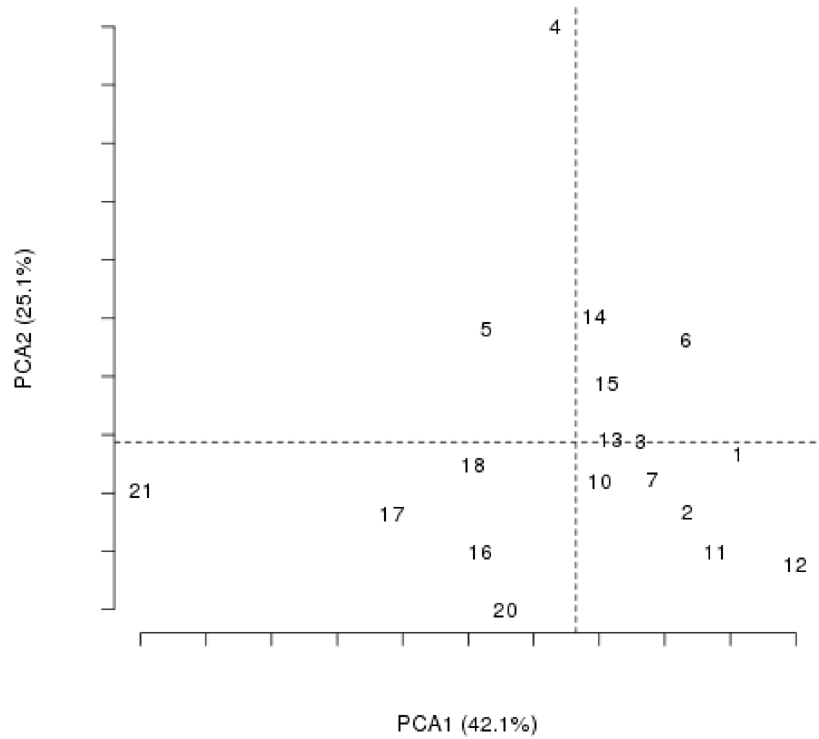

Figure 2. Biplot representation of the sites by inbred interaction analysis using the additive main effects and multiplicative interaction model. This illustrates the first versus second principal components for the cured leaf weight in the Virginia varietal group during the 2009-2010 season.

CONCLUSIONS

Our results are very similar for both tobacco varietal groups. There is no advantage of using a large number of sites for VCU experiments in the southern region of Brazil. Many sites contributed little to the interaction or did not allow the lines to be discriminated. Using a few sites, the classification of the best lines was very similar to that obtained using the total number of evaluated sites.

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