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ARTICLE

Exploitation of heterosis in tobacco breeding in Brazil

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Abstract: *This study estimated the general and specific combining abilities and mean heterosis of tobacco lines of the varietal groups Flue-Cured Virginia (FCV) and Air-Cured Burley (BY). In addition, possible advantages and an improved strategy of using hybrids in tobacco breeding in the tropics were discussed. Ten BY and 13 FCV lines were crossed in a diallel mating design. The hybrids and parental lines were evaluated at two locations in Southern Brazil. The mean heterosis was 5.37% for FCV and zero for BY lines, although higher than 15% in some combinations. The occurrence of heterosis indicates dominance in the control of tobacco yield. Tobacco breeding programs must focus primarily on improving the performance of lines per se and then on the identification of the best hybrid by testing combinations.*

Keywords: *Plant breeding, quantitative genetics, diallel crosses, hybrid vigor*

INTRODUCTION

Tobacco (*Nicotiana tabacum* L.) is the non-food crop with the highest economic value in the world, with an annual production of more than 5.9 million tons (http://www.afubra.com.br). Brazil is the second largest producer, accounting for approximately 11.5% of the global output, and tobacco is one of the 10 agribusiness products that generate the highest export revenue for the country (FAO 2018). Ninety-two percent of Brazilian tobacco is produced on family farms in the three southern states Paraná, Rio Grande do Sul, Santa Catarina, where this activity is the main source of income. Of the different cultivated varietal groups, the two most important are Flue-Cured Virginia (FCV), which accounts for 83% of the production, and Air-Cured Burley (BY), with 12% (http://www.afubra.com.br).

For the main autogamous species, the key objective of breeding programs has been to commercialize pure line cultivars. Hybrids are however used with a view to combining simply inherited traits into single genotypes, rather than as a means of exploiting true heterosis, as is done in tomato for example (Tamta and Singh 2018). For tobacco, the use of hybrid seeds should be considered from another angle, because artificial crosses are already routinely made to maintain the isogenic male sterile line and protect the cultivars, so that extra cost with hybrid crosses could be avoided (Schnable and Wise 1998).

Since an artificial mating design is used to produce commercial tobacco seed, the possibility of improving tobacco performance by exploiting heterosis should be considered and the most appropriate strategy for the production of

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hybrid seeds chosen. To this end, the general and specific combining abilities of the lines would have to be estimated. The additional work of breeders would be to identify lines that complement each other when crossed at a determined stage of the breeding program. In other words, to use hybrids, line complementarity should be assessed based on estimates of combining ability.

Diallel mating designs are often used when a series of lines are part of a breeding program and superior combinations have to be identified (Hallauer et al. 2010, Rocha et al. 2018). This methodology is already being applied to several species (Bignotto et al. 2015, Barata et al. 2019) and has been described for tobacco in temperate climate. However, in Brazil, this type of data has not been compiled and results may be different for the tropics.

This study estimated the general and specific combining abilities of lines of the varietal groups FCV and BY, as well as the mean heterosis, and discussed which breeding strategy would be best for the inclusion of hybrids in tobacco breeding programs in the tropics.

MATERIAL AND METHODS

The evaluated Flue-Cured Virginia (FCV) and Air-Cured Burley (BY) tobacco lines were the best-performing of the British American Tobacco (BAT) breeding program. The 13 selected FCV lines were crossed in a diallel mating design, where 72 of the 78 possible combinations were established. The 10 BY lines were selected and crossed in a diallel mating design, where all 45 combinations were established.

The diallel crosses of the FCV group were evaluated at two locations in southern Brazil. The first location was an experimental research station, in Mafra (lat 26^0 10’ 00’’ S, long 49^0 48’ 59’’ W, 848 m asl), Santa Catarina and the second a private tobacco farm in the same district (lat 26^0 06’ 50’’ S, long 49^0 56’ 13’’ W, 801 m asl). The BY group was assessed at the experimental station described above and on a private farm of BY tobacco in Campo do Tenente (lat 26^0 06’ 33’’ S, long 49^0 56’ 51’’ W, alt 802 m asl), Paraná.

The seeds were sown in a greenhouse in early July and planted in the field in early October. The parental lines, hybrids, and checks of FCV were evaluated in a 10 x 10 triple lattice and those of BY in an 8 x 8 triple lattice. Each plot consisted of one row with 10 plants, at a spacing of 1.2 m between rows and 0.5 m between plants for FCV, and 1.15 m between rows and 0.45 m between plants for BY. The crop was managed as recommended for commercial tobacco production in the respective growing regions for each varietal group. Yield (YLD, kg ha^{-1}) was determined by weighing the total leaf quantity after curing.

With the data, variance analysis was performed for each location and then combined analysis, using a procedure similar to that proposed by Ramalho et al. (2012a). For combined variance analysis, the source of variation among treatments was partitioned in parental hybrids (H), parent lines (L) and checks (C), hybrids vs parental lines (H vs L) and hybrids+parental lines vs checks (H+L vs C). In the analysis, the treatments, locations and mean effects were considered fixed and the replication effect random.

Additionally, with the means of the two locations, diallel analysis was carried out based on Griffing II (1956) without reciprocals, according to the model:

$$Y_{ij} = m + g_i + g_j + s_{ij} + \bar{e}_{ij}$$

where Y_{ij} is the mean value of the hybrid combination ($i \neq j$) of the parent (i = j); m is a constant that represents the overall means of the treatments of the diallel; g_i and g_j are the effects of the general combining ability of the $i - eth$ and $j - eth$ parent, respectively; s_{ij} is the effect of the specific combining ability for the cross between parents i and j; and e_{ij} is the mean experimental error of both locations, $\bar{e}_{ij} \cap N(0, \sigma^2)$. All data analyses were performed using R software (R Development Core Team 2021).

Heterosis (H) was estimated from the means of the treatments involved in the diallel. The heterosis of each hybrid combination H_{ij} (%) was estimated by:

$$H_{ij} = (F_{1ij} - (l_i + l_j)/2)/((l_i + l_j)/2) \times 100$$

where: F_{1ij} is the hybrid combination between i and j and the parental line mean $l_i + l_j$.

Mean heterosis ($\bar{H}_{(\%)}$) was calculated by the equation (Ramalho et al. 2012b):

$$\bar{H}_{(\%)} = \frac{(\bar{H} - \bar{P})}{\bar{P}} \times 100.$$

where $\bar{H}$ is the mean F_{1ij} of all hybrids and $\bar{P}$ the parent mean of all treatments in the diallel. In the combined analysis of the source of variation treatments, the contrast parents *vs* hybrids was used to check if mean heterosis was different from zero.

RESULTS AND DISCUSSION

The coefficients of variation (CV) were below 15% for both varietal groups and close to those of other studies on tobacco (Pulcinelli et al. 2014). Although the CV was low, the accuracy was medium. In such situations, it is concluded that the Mean Square Error (MSE) as well as the Mean Square Treatment (MST) are relatively low. As accuracy was computed by the estimator: $r_{gg'} = \sqrt{1 - \frac{1}{F}}$, the difference between the MSE of the locations was low. Under this condition, combined analysis was performed without restrictions (Table 1).

Combined variance analysis (ANOVA) was significant for the source of variation in location (SV) ($P<0.01$); the trait means of each environment can explain this difference. The mean of location 2 was 12.4% higher for FCV and 15.0% higher for BY at location 1 (Table 1).

The SV in treatment was significant ($P<0.01$) for both varietal groups. Significant differences ($P<0.01$) in all cases were also observed when the treatment SV was partitioned in hybrids (H), parents (L) and checks (C). The contrast between H vs L, which assesses whether mean heterosis is equal or different from zero, was only significant ($P<0.01$) for FCV, because $P=0.82^{ns}$ for BY lines. For FCV, the hybrid mean was 5.34% higher than that of the lines, i.e., mean heterosis was 5.34% (Table 1).

The contrast H+L vs C was also significant ($P<0.01$) in both varietal groups. The hybrid mean was superior to the check mean (10.7% for FCV, 16.4% for BY), indicating the potential of the tested lines and hybrids in relation to commercial controls (Table 1).

The interaction treatment SV x location (TxA) was significant ($P<0.01$). The occurrence of genotype x environment interaction is common in tobacco (Pulcinelli et al. 2014) and other species (van Eeuwijk et al. 2016). In case of an interaction, there are two options: the results can be taken into consideration separately for each environment or for the mean of the environments. In this study, only two environments/locations were evaluated, which do not necessarily represent all tobacco growing conditions of southern Brazil. Moreover, the future climatic conditions are unknown. Therefore, since the purpose of this study was to obtain heterosis estimates that are as accurate as possible, it was decided to use the mean data of both locations. This strategy has frequently been used elsewhere (Lima et al. 2014, Ferreira et al. 2015, Mendes and Ramalho 2018).

Table 1. Estimates of the coefficient of variation, accuracy (Ac), homoscedasticity and mean parameters in each environment for tobacco yield (YLD, kg ha^{-1})

Location	Parameter	FCV	BY
1	CV (%)	12.23	9.29
	Ac	0.49	0.54
	Mean	3490	2318
2	CV (%)	14.85	11.38
	Ac	0.46	0.67
	Mean	3922	1995
Overall mean		3706	2156
Parent mean (G)		3598	2243
Hybrid mean (H)		3791	2234
Diallel mean		3761	2235
Check mean		3399	1920
Mean heterosis (%)		5.37	(-0.40)
Homoscedasticity*		1.35	1.05

* Homoscedasticity: Mean square error - maximum (MAX) divided by the minimum (MIN).

For the combined environments, diallel analysis detected differences between the lines ($P<0.01$) for general combining ability (GCA) of both varietal groups, in contrast to the specific combining ability (SCA) SV ($P=0.27$ for FCV and $P=0.13$ for BY). Some studies on tobacco in the literature report that the contribution of GCA is much higher than that of SCA (Matzinger et al. 1962). The same is frequently observed for other autogamous species apart from tobacco (Rios 2015).

It is however noteworthy that although SCA did not differ significantly, it explained most of the variation in the diallel (69.5% for FCV and 55.6% for BY) (Table 2). Similarly, SCA explained most of the variance without being significant because the SCA source of variation was associated with a higher number of degrees of freedom than the GCA source of variation (SV). Thus, the MS of SCA was lower than that for GCA. However, the importance of SCA, in the case of dominance for the trait dry matter of cured leaves, should not be neglected to explain the differences in hybrid performance of FCV and BY lines.

Table 2. Estimates of sum squares of SCA and GCA for cured leaf weight (YLD, in kg ha^{-1})

Parameter		FCV	BY
GCA	df	78	38
	SS	26149400	3206164
SCA	df	12	9
	SS	11442553	2562808
$[MS_{SCA}/(MS_{SCA} + MS_{GCA})]*100$		69.56	55.58

Table 3. Mean estimates of the varietal groups FCV and BY and GCA for cured leaf weight (YLD, in kg ha^{-1})

Line	Varietal Group			
	FCV		BY	
	MEAN	YLD	MEAN[1]	YLD1[1]
1	3461.83	68.49	2418.67	105.85
2	4114.50	163.01	2327.67	117.33
3	3836.33	-32.71	2247.83	-42.76
4	3067.67	-66.80	2183.00	2.14
5	3060.17	-136.30	1853.00	-90.14
6	4162.50	39.62	2268.83	-1.41
7	3517.83	-237.04	2261.17	51.01
8	3812.00	95.25	-	-76.24
9	3539.67	18.76	-	43.88
10	3752.83	8.84	-	-109.66
11	4025.67	-30.55	-	-
12	3297.50	-17.97	-	-
13	3581.17	127.41	-	-
$s_{\hat{g}_i}$	-	61.13	-	37.81
$s_{\hat{g}_i - g_j}$	89.98	-	56.36	

[1] Lines 8, 9, and 10 for BY were not included in the field trial. Respective errors associated with the estimates of each parent and the between-parent comparison ($s_{\hat{g}_i}$ and $s_{\hat{g}_i - \hat{g}_i}$).

The mean performance data of lines and estimates of general combining ability ($\hat{g}_i$) are shown in Table 3. Note that the correlation between means and $\hat{g}_i$ estimates is not high, in particular for FCV ($r_{FCV} = 0.48$). If there were no dominance, the correlation estimated between the mean of the parents and $\hat{g}_i$ would be high. In this situation, hybrid performance would only be calculated as a function of the mean of the parental lines, i.e., without heterosis. Model-II of Griffing (1956) for diallel analysis proves this fact:

$$Y_{ij} = \overline{Y} + \hat{g}_i + \hat{g}_j + \hat{s}_{ij}$$

Thus, the difference between the hybrids ($Y_{ij} - \overline{Y}$) detected by analysis of variance, as already mentioned, would be due to GCA and SCA, disregarding the occurrence of experimental error. An explanation for what $\hat{g}_i$ represents can be established based on what happens for one locus B (Vencovsky and Barriga 1992):

$$\hat{g}_i = (p_i - \overline{p})[a_B + (1 - 2\overline{p})\, d_B]$$

where p_i and $\overline{p}$ are the allelic frequencies of parent *i* and the parent mean, respectively; a_B and d_B are the deviations of the homozygous and heterozygous contribution, respectively, in relation to the homozygous mean for each locus. In other words, if $\overline{p}$ =0.5 or d = 0, the line mean would fully explain the combining ability and there would be no need to test the lines in hybrid combinations. In this study, for the FCV lines it was clear that the additive effect alone was not sufficient to explain $\hat{g}_i$, because the three highest-yielding parents were lines 2, 6 and 11; but of these three, only parent 2 was among those with highest $\hat{g}_i$ (Table 3). This is a strong indication that the second part of the expression also contributed to $\hat{g}_i$ and therefore, d was different from zero and/or $\overline{p} \neq 0.5$. This is the case because in the diallel, $\overline{p}$ is almost always different from 0.5.

Considering that $\hat{s}_{ij}$ also contributed to the performance of the hybrid combination and only one locus B, $\hat{s}_{ij}$ was computed by the following expression (Venkovsky and Barriga 1992):

$$\hat{s}_{ij} = 2[(\overline{p} - p_i)(r_i - \overline{r})d_B]$$

where r_i and $\overline{r}$ have the same significance as p_i and $\overline{p}$, but for the other parent. This expression clearly showed that $\hat{s}_{ij}$ will only be different from zero in case of parental divergence and dominance in the trait control. The results of this study reinforced the importance of SCA in explaining the total variation among the hybrids (Tables 2 and 3). This fact is rather uncommon in autogamous species.

Proof of this fact are the estimates of the combining abilities of the two parents ($\hat{g}_i$ and $\hat{g}_j$) and $\hat{s}_{ij}$ of the 10 best hybrids of the varietal group FCV (Table 4) and BY (Table 5). Note that for the best FCV hybrid (2x3), the heterosis estimate was not the highest (9%). However, both GCA and SCA were positive. Hybrid combinations with the highest heterosis means (15%) were the combinations 1x8 and 2x5. In these two cases, $\hat{s}_{ij}$ was higher than $\hat{g}_i$'s. Interestingly, 30% of all hybrid combination derived from FCV lines (Table 4) had a lower mean than the parental lines, i.e., negative heterosis. In the case of BY, heterosis of 15% of the hybrids was negative. In this case, even among the 10 hybrids with highest means, six had negative heterosis estimates (Table 5).

When *H* is only a function of dominance (d), positive or negative values can be obtained, depending only on the d direction. Nevertheless, when positive and negative values are found for the same traits, d alone is not sufficient to explain the superiority of some hybrid combinations (Wen et al. 2016). Additive x additive epistasis, which is common in autogamous plants, has been one of the hypothetical explanations for the occurrence of heterosis (Bernardo 2020, Ginkel and Ortiz 2018). This could be the reason that explains the occurrence of positive and negative heterosis simultaneously in the same diallel, as was the case in this experiment.

The magnitude of heterosis in autogamous plants is normally not the same as that of alogamous plants (Chen 2010, Bernardo 2020). In autogamous species, where selfing occurs naturally, the frequency of deleterious alleles is lower, possibly a result of evolution. As a consequence, inbreeding depression is low or close to zero (Allard 1999). The mean heterosis in this study was close to that reported in other autogamous species (Bernardo 2020). Heterosis in tobacco has also been assessed in other countries, for traits related to yield and alkaloid accumulation, and estimates comparable to those of our study were found (Vandenberg and Matzinger 1970, Aleksoski 2010). At least in principle, heterosis reported in temperate climate is similar to that found in the tropics.

It should be noted that hybrids can only be commercialized when the hybrid seed can be produced at a cost that is consistent with the superior agronomic performance. In other words, the feasibility of commercial F_1 hybrids depends on the observed heterosis (Gupta et al. 2019). In tobacco, the use of hybrid seeds should be considered from another angle, as mentioned above, because artificial crosses are already routinely made to protect the cultivars by male sterility and so no extra cost is involved. Thus, even at low levels of heterosis for yield, hybrids must be purchased, as mentioned above. It must be pointed out that the performance of some parental lines (parent 6 in FCV and parent 1 in BY) was better than that of some hybrids (Tables 3, 4 and 5). This result was expected, as the mean heterosis was low. It is also worth mentioning that the objective of this study was not to identify the best parental lines or hybrids for commercialization but rather to estimate heterosis and discuss the best future strategy for the breeding program. The only trait assessed

Table 4. Means of two locations of the 10 highest-yielding hybrids (YLD, in kg ha^{-1}), and respective specific heterosis estimates (%), $\hat{g}_i$, $\hat{g}_j$ and $\hat{s}_{ij}$ for the varietal group FCV

Hybrid	YLD	Heterosis	$\hat{g}_i$	$\hat{g}_j$	$\hat{s}_{ij}$
3x2	4346	9	123.5	176.9	271.6
11x7	4224	12	-15.2	4.9	460.1
8x1	4194	15	91.3	-17.8	346.0
9x2	4163	9	-36.6	176.9	248.3
8x3	4134	8	91.3	123.5	145.5
5x2	4130	15	-136.2	176.9	315.6
6x3	4116	3	64.4	123.5	153.8
10x7	4105	13	36.7	4.9	289.0
1x2	4097	8	-17.8	176.9	164.1
3x12	4057	14	123.5	17.5	142.4

Table 5. Means of two locations of the 10 highest-yielding hybrids (YLD, in kg ha^{-1}), and respective specific heterosis estimates (%), $\hat{g}_i$, $\hat{g}_j$ and $\hat{s}_{ij}$ for the varietal group BY

Hybrid	YLD	Heterosis	$\hat{g}_i$	$\hat{g}_j$	$\hat{s}_{ij}$
2x4	2538	13	117.3	2.1	183.7
2x9	2460	-	117.3	43.9	64.7
2x6	2432	6	117.3	-1.4	81.7
2x3	2410	5	117.3	-42.8	101.1
6x9	2392	-	-1.4	43.9	114.8
1x9	2387	-	105.8	43.9	2.7
5x7	2380	16	-90.1	51.0	184.6
1x8	2379	-	105.8	-76.2	114.8
7x9	2372	-	51.0	43.9	42.9
1x10	2351	-	105.8	-109.7	120.8

here was tobacco yield (YLD), but despite the priority of this characteristic, there are others such as disease resistance and quality aspects that should be taken into consideration for selection.

It is clear that the mean hybrid performance depends on the good performance of the lines, because in the mean of all hybrids evaluated, the line performance *per se* was responsible for 94.63% of the mean and heterosis for only 5.37%. Once more, this reflects the presence of heterosis in the two tobacco varietal groups; although low, it should be exploited in commercial hybrids instead of lines. Based on the above, to breed good hybrid tobacco cultivars, breeding programs must initially identify lines with a good performance *per se* (CGA) and then those that complement each other best to ensure maximum exploitation of heterosis (SCA). Thus, after establishing the lines, crosses must be performed in a diallel mating design to breed the best hybrid cultivars.

CONCLUSION

The mean heterosis for FCV was 5.37% and for BY lines zero, although higher than 15% in some combinations. The occurrence of heterosis indicates the presence of dominance in the control of tobacco yield, as shown by the SCA effect.

Tobacco breeding programs must initially focus on improving the performance of lines *per se* and then testing the combinations between the best lines to identify the best hybrid.

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