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Feasibility of using tobacco hybrids of the Dark tobacco type

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ABSTRACT. The species *Nicotiana tabacum*, known as tobacco, is one of the crops with the highest economic value in the world among non-food species. Since 2000, Brazil has become the world's second largest tobacco producer. Brazilian production is mainly concentrated in the South region. The main tobacco types are produced in Brazil, such as Dark, which, despite representing a small percentage of the total production in Brazil, is economically important because of a high added value. Until now the only available cultivar is a line that despite having a desirable alkaloid content, has low productivity. In an effort to help improve production, we estimated the heterosis of diallel crosses involving tobacco lines of the Dark tobacco type. A complete diallel cross was made involving 10 lines from the British American Tobacco company breeding program. The 45 hybrid combinations, together with the 10 parental lines and nine other commercial controls, were evaluated at four sites in Southern Brazil, using a triple lattice experimental design (8 x 8). The characteristics evaluated included: green leaf mass (Productivity - YLD), and total alkaloid content (ALK), and the selection index was obtained by the sum of standardized variables (SSV), considering a weight of 70% for YLD and 30% for ALK. Estimated correlation between YLD and the ALK was -0.66. The use of the selection index allowed simultaneous gains in both traits, although they were of less magnitude than selection for each characteristic individually. The average heterosis was 8.6% for productivity, -1.4% for the total alkaloid content, and 5.3% for the selection index. However, considering the selection index, hybrids were obtained with an average heterosis greater than 10%. Under these conditions, the use of hybrids should be encouraged not only to combine favorable phenotypes, but also to explore the beneficial effects of heterosis.

Key words: *Nicotiana tabacum*; Heterosis; Diallel; Plant breeding

INTRODUCTION

The species *Nicotiana tabacum*, known as tobacco, is one of the crops with the highest economic value in the world among non-food species. Since 2000, Brazil has become the world's second largest tobacco producer, second only to China; however, it is the world's largest exporter. Brazilian production is mainly concentrated in the South region, which accounts for 98% of total production, where about 557 municipalities are involved in tobacco production, generating more than two million jobs (Kist et al., 2020).

The main tobacco types cultivated in Brazil and in the world are Virginia and Burley. However, there are other tobacco types such as Dark, which despite representing a small percentage of the total production, are economically important because they have a high added value. Though Dark tobacco has other uses, the leaf produced in Brazil is mainly used to manufacture "Snus", which are tobacco sachets for oral ingestion. This form of consuming tobacco is considered less toxic and harmful than cigarettes (Clarke et al., 2019). Sweden, Norway, and the United States are the main consumers.

All Dark tobacco produced in Brazil is exported. A major crop problem is that the main cultivar available is the line 'Dark OS' which, despite having the desirable alkaloid content, has low productivity. The breeding program of this tobacco type has been running for some years and promising inbred lines have been identified. However, the potential of these lines for hybrid combinations is unknown. Diallel crosses are widely used to assess the potential of lines to obtain hybrids (Bernardo, 2020). There are some methods to analyze this type of cross design; the Gardner and Eberhart (1966) procedure is the most suitable to obtain heterosis information.

To protect their germplasm, tobacco companies use cytoplasm sterility. In this way, the seeds are produced annually using isogenic, male-fertile and male-sterile lines (Chen and Liu, 2014). In other words, there is the same work of obtaining a hybrid, without, however, exploring heterosis. What is questioned is whether it would be feasible or not to obtain hybrids that complement each other in some attributes. However, information on the existence or not of heterosis in the crossing of the Dark tobacco type lines was not found in the literature. For other tobacco groups, there are reports that heterosis occurs, and on average it does not exceed 8% for yield; however, certain combinations have more expressive heterosis values (Dexter-Bone and Lewis, 2019). Similar results have been reported in other autogamous species (Labroo et al., 2021).

In the case of the Dark tobacco, two characteristics are important, the yield and the content of alkaloids, which normally must be superior to the traditional Virginia and Burley groups. Thus, in the selection of new lines or, mainly, when obtaining hybrids, both characters must be considered. Using a selection index is the best option when more than one trait should be selected (Ramalho et al., 2012; Bernardo, 2020). The index to be used must be easy to estimate and interpret and obtained with less possibility of error. In this context, the index of the sum of standardized variables (SSV) is a good alternative (Ramalho et al., 2012; Lima et al., 2015).

From the above, this research was conducted to estimate the heterosis of the traits green leaf mass (productivity - YLD), total alkaloid content (ALK), and the SSV index, considering simultaneously the YLD and ALK, in a diallel cross involving lines from the Dark tobacco group, in order to verify the possibility of using hybrids.

MATERIAL AND METHODS

The data used for this paper were provided by the company British American Tobacco (BAT) Brazil. Four locations were considered to assess the experiments, three in the region that represents the commercial production of Dark Tobacco and one in the company's experimental area, in Mafra, which is a traditional area for tobacco production in Brazil, though it is not a region that produces this type of tobacco (Table 1).

This study was carried out using a diallel of 10 parental lines from the BAT Breeding Program belonging to the Dark tobacco type. The 45 hybrids from the diallel were evaluated together with the 10 parental inbreed lines and nine other commercial controls in the 2016/17 crop season. The design used was the triple lattice (8 x 8). The plots consisted of one row with ten plants, spacing 1.4 m between-row and 0.7 m between plants. External borders were used in the experiment with a commercial cultivar. The management of tobacco farming, such as seedling production, transplanting, and conducting the research were those recommended by the company's agricultural technological package. The harvest was carried out by harvesting the entire plant at once, as is commonly done for this tobacco type. It was estimated the green leaf mass (productivity $kg.plot^{-1}$) - "YLD" and later in the laboratory the total alkaloids content (%) - "ALK".

Individual variance analyses were performed initially for each trait. Considering that the lattice design did not present superior efficiency to the randomized blocks, in any of the locations, it was decided to proceed with the analysis of variance using the randomized blocks model. Subsequently, joint analyses of variance were performed.

The YLD and ALK data were standardized for each repetition. Since the standardized values are deviations around the zero average, negative and positive values occur and to allow the analysis of variance to be carried out, the constant '5' was added. Thus, the average became five. At this time, a selection index was constructed involving the two variables. Each trait was given a weight based on its economic and commercial importance, which was 70% for YLD, and 30% for ALK.

Using the averages of the isolated traits and the index, a diallel analysis was carried out according to the model of Gardner and Eberhart (1966): $Y_{ij} = m + (p_i + p_j)/2 + \theta(h + h_i + h_j + s_{ij}) + e_{ij}$, where Y_{ij} is the average value observed in a parent (i = j) or in a hybrid combination ($i \neq j$); m: average of parental populations; p_i: effect of parental i; p_j: effect of parental j; $\theta = 0$, when i = j e $\theta = 1$, when $i \neq j$; h: effect of medium heterosis; h_i: effect of parental heterosis i; h_j: effect of parental heterosis j; s_{ij}: effect of specific heterosis; and e_{ij}: average experimental error.

The analyses were carried out using the Genes program (Cruz, 2006). The estimates of heterosis in relation to the average of parents (h%) were obtained by the following estimator: $h\% = \frac{(\overline{h_{ij}} - \frac{\bar{P}_i + \bar{P}_j}{2}) * 100}{(\frac{\bar{P}_i + \bar{P}_j}{2})}$, where h_{ij} is the average of the hybrid ij and P_i and P_j are the average of parental i and j. Using the averages of the four locations, the phenotypic correlation between the YLD and the ALK was estimated (Ramalho et al., 2012).

RESULTS

The Dark tobacco cultivation region in Brazil is located in the Southwest region of the state of Paraná. In these locations, the climate is warm and wet. There is significant rainfall throughout the year and even the driest month has rainfall (Table 1). The yield and total alkaloid content are highly influenced by the climatic conditions of the region where the tobacco is grown (Tang et al., 2020). The mean of green leaf mass (YLD) in the three locations in the producing region was 24.96 $kg.plot^{-1}$ (2371 $kg.ha^{-1}$) and they did not differ from each other. This value was 19.5% higher than that obtained in the company's experimental area, where this tobacco type is not cultivated commercially. For total alkaloid content (ALK), the same occurred, the mean of the three locations was 3.34%, exceeding the average obtained in the experimental farm by 26%. This type of tobacco is well adapted to conditions in Southwest Paraná state.

Table 1. Characteristics of the four locations where the experiments were carried out to assess the diallel crossing of Dark tobacco.

LOCATION	1	2	3	4
City	Serranópolis do Iguaçu – PR[1]	São Miguel do Iguaçu - PR	São Miguel do Iguaçu – PR	Mafra - SC
Altitude (m)	233	248	269	840
South Latitude	25° 23'46.0"	25°37'03.7"	25°15'49.7"	26º10'00.8"
West Longitude	054°05'44.1"	054°29'.49.1"	054°12'95.2"	049º47'58.2"
Average annual temperature	19.8ºC	20ºC	20ºC	17.3ºC
Average annual rainfall	1837 mm	1755 mm	1755 mm	1288 mm

[1]PR: Paraná State, SC: Santa Catarina State.

In the analysis of variance of the traits YLD and ALK, the estimates of the accuracy of the experiments, in each location, for YLD were all above 0.94 and above 0.86 for ALK (data not shown, available from the authors upon reasonable request), indicating good experimental precision. A significant difference ($p<0.01$) was detected between the treatments in all locations, a condition that is indispensable to achieve the objectives of the research proposition. In the joint analysis, the source of variation (SV) Treatments and Locations was significant ($p<0.01$), and the same occurred with the interaction Genotypes x Environments (GE). Similar results were obtained for the selection index (data not shown, available by the authors upon reasonable request).

In the decomposition of the SV Treatments, the contrast between the treatments of the Diallel (Hybrids + Parental) *vs.* Contr was significant ($p<0.01$). The average of Hybrids + Parental was 22.2% higher than the controls, when the index was considered (data not shown, available from the authors upon reasonable request).

The average YLD of the parental varied from 15.64 $kg.plot^{-1}$ (1485 $kg.ha^{-1}$) (Line 6) to 28.67 $kg.plot^{-1}$ (2724 $kg.ha^{-1}$) (Line 4) (Table 2). In the case of ALK the variation was 2.53% (Line 8), to 4.05% (Line 6) (Table 3). It appears that only parental 6 and 7 showed a percentage of alkaloids within the desirable interval for this tobacco type. These results are agreeing with the negative association observed between ALK and YLD ($r = -0.66$), which is an additional complicating factor in obtaining hybrids that associate high YLD and ALK.

Table 2. Means of green leaf mass (YLD) ($kg.plot^{-1}$) of lines (parental) (diagonal) and hybrids (above diagonal), heterosis in relation to the average of lines (%) (below diagonal), parental heterosis (H_i) and the average heterosis (h). Data obtained from a diallel with 10 lines of the Dark tobacco type. Assessments conducted at four locations.

Parents	1	2	3	4	5	6	7	8	9	10	H_i
1	**27.20**	28.21	28.15	30.63	28.98	21.41	25.45	28.42	28.38	25.26	0.00
2	10.42	**23.90**	26.57	29.35	25.96	20.22	25.74	25.63	27.41	24.20	0.20
3	8.03	8.89	**24.91**	29.18	27.52	22.69	25.80	27.82	28.21	23.25	0.43
4	9.84	11.86	9.12	**28.57**	27.62	22.58	26.66	27.27	28.63	26.77	-0.21
5	15.18	10.40	14.60	6.86	**23.13**	21.28	24.06	25.11	28.29	25.50	0.72
6	-0.04	2.28	11.92	2.14	9.77	**15.64**	18.94	20.08	22.76	21.64	-0.88
7	4.25	13.10	10.90	6.23	7.53	1.63	**21.62**	25.74	26.20	24.32	0.04
8	11.30	7.35	14.09	4.02	6.90	1.68	13.18	**23.86**	25.93	24.44	-0.14
9	7.26	10.49	11.45	5.49	15.87	10.07	10.74	4.62	**25.71**	23.94	0.10
10	4.06	6.96	0.53	7.25	14.67	17.00	13.19	8.10	1.75	**21.35**	-0.27
	$\bar{h}$	2.016	(8.6%)								
$s^2(H_i)$**		**0.167**									
$s^2(H_i - H_j)$		0.371									

* Error associated with parental heterosis estimate.

Table 3. Means of total alkaloid content (ALK) (%) of lines (parental) (diagonal) and hybrids (above diagonal), heterosis in relation to the average of lines (%) (below diagonal), parental heterosis (H_i) and the average heterosis (h). Data obtained from a diallel with ten lines of the Dark tobacco type. Assessments conducted at four locations.

Parents	1	2	3	4	5	6	7	8	9	10	H_i
1	**3.12**	3.01	3.04	2.87	3.08	3.65	3.30	2.75	2.63	2.82	-0.08
2	-2.87	**3.09**	3.07	2.69	3.23	3.43	3.39	2.94	2.64	2.85	-0.05
3	-7.17	-5.63	**3.43**	3.11	3.42	3.52	3.31	3.08	2.80	2.79	-0.11
4	-4.93	-10.37	-1.99	**2.92**	3.03	3.30	3.19	2.90	2.83	2.73	-0.04
5	-3.89	1.50	1.84	-2.23	**3.28**	3.54	3.58	2.87	3.03	3.12	0.06
6	1.89	-3.70	-5.78	-5.32	-3.52	**4.05**	3.88	3.64	3.54	3.55	0.07
7	-2.57	0.60	-6.50	-2.90	3.24	0.59	**3.66**	3.04	3.01	3.10	-0.02
8	-2.75	4.84	3.48	6.48	-1.29	10.85	-1.77	**2.53**	2.78	2.76	0.17
9	-8.76	-7.96	-7.80	1.79	2.23	5.91	-4.59	7.65	**2.64**	2.56	-0.01
10	-2.87	-1.40	-8.83	-2.74	4.51	5.23	-2.52	5.56	-4.16	**2.69**	0.02
	$\bar{h}$	-0.043	(-1.4%)								
$s^2(H_i)$*		0.003									
$s^2(H_i - H_j)$		0.008									

* Error associated with parental heterosis estimate.

How should breeders proceed in a situation like this? The most advisable is to obtain a selection index (Resende et al., 2014; Bernardo, 2020). An important decision when setting the index is the choice of weights for each trait (Céron-Rojas and Crossa, 2018; Bernardo, 2020). In the case of tobacco, YLD is extremely important, as Dark tobacco producers have been requiring the increase their income and one of the ways to achieve this goal is to increase the amount of tobacco produced per hectare, which guarantees the sustainability of tobacco production.

Another very important characteristic in Dark tobacco, as already mentioned, is the concentration of total alkaloids (nicotine). Dark tobacco is mainly intended to manufacture sachets with gradual release of nicotine, with the average values of alkaloids in the plants of this type of tobacco ranging from 3.5to 4.5%; therefore, being on average higher than other tobacco groups such as Virginia and Burley. Please note that this is an average range, since the alkaloid rates can vary depending on the positions of the leaf in the plant, the level of maturity in the harvest, among other factors (Lewis, 2019; Henry et al., 2019). Regarding these considerations, the option was to use the Sum of Standardized Variables index (SSV) and the weights of 70% for YLD and 30% for ALK.

Concerning to the index (SSV), the parental with performance above average were numbers 1, 3 and 4 (Table 4). Parental 1 was among the highest average for YLD and ALK. Parental 3 also presented good performance, especially for ALK. Parental 4 showed a high SSV value, mainly due to the higher average of YLD, since ALK was the lowest among the evaluated parentals (Tables 2, 3 and 4).

Table 4. Means of the selection index (SSV) of lines (diagonal) and hybrids (above diagonal), heterosis in relation to the average of lines (%) (below diagonal), parental heterosis (H_i) and the average heterosis (h). Data obtained from a diallel with 10 lines of the Dark tobacco type. Assessments conducted at four locations.

Parents	1	2	3	4	5	6	7	8	9	10	H_i
1	**5.40**	5.52	5.52	5.76	5.64	4.93	5.30	5.38	5.31	4.99	-0.04
2	6.80	**4.93**	5.31	5.46	5.33	4.66	5.38	5.11	5.19	4.85	0.01
3	3.33	4.00	**5.28**	5.67	5.66	5.04	5.35	5.49	5.38	4.69	0.00
4	5.78	4.75	5.40	**5.49**	5.44	4.90	5.39	5.31	5.46	5.15	-0.05
5	8.93	7.90	).70	4.15	**4.95**	4.84	5.27	4.99	5.54	5.20	0.14
6	1.11	0.53	4.77	-0.19	4.26	**4.34**	4.72	4.73	5.06	4.88	-0.08
7	2.06	8.51	4.26	2.93	6.04	1.21	**4.98**	5.19	5.22	5.02	-0.02
8	7.26	7.00	).96	5.03	4.25	5.65	8.02	**4.62**	5.06	4.85	0.08
9	2.35	4.80	4.82	4.34	11.58	8.71	4.77	5.44	**4.98**	4.68	0.00
10	1.93	4.28	2.99	4.32	11.57	12.00	7.14	7.74	0.07	**4.38**	-0.03
$\bar{h}$	0.2604	5.3%)									
$s^2 (H_j)$*	0.0048										
$s^2 (H_i - H_j)$	0.0106										

* Error associated with parental heterosis estimate.

In the diallel analysis, it was noted that the SV heterosis was significant (p<0.01) for the traits individually (data not shown, available from the authors upon reasonable request). In the decomposition of this SV, the average heterosis was significant (p<0.01) only for YLD. However, the parental heterosis was not significant for any of the traits. It is inferred that the parental lines equally contributed to the heterosis. Regarding to the specific heterosis, this SV was significant ($P \leq 0.05$) for all analyzed traits. For the SSV, only a significant difference was not found for parental heterosis FV.

When the YLD of the 45 hybrids obtained was assessed, it was observed that the best hybrid for YLD was 1x4 (30.63 $kg.plot^{-1}$). However, note that this combination was not the one with the highest estimate of heterosis ($\bar{h}_{\%}$). Even so, the $\bar{h}_{\%}$ of this hybrid was above the average heterosis (8.6%). The average heterosis was -1.4% for ALK (Table 3). The 6x7 hybrid had the highest average (3.88%), although also not the highest estimate of $\bar{h}_{\%}$ (0.59%). Regarding YLD, the $\bar{h}_{\%}$ estimates ranged from -0.04% (Hybrid 1x6) to 17.00% (Hybrid 6x10) (Table 2). For ALK, the variation was from -10.37% (Hybrid 2x4) to 10.85% (Hybrid 6x8) (Table 3). Note that for both traits, the hybrids with the best performance always presented positive parental heterosis (H_i) estimates (Tables 2 and 3). No reference regarding the contribution of heterosis was found for the Dark tobacco type. However, the heterosis estimate for YLD was similar to that obtained for the FC Virginia tobacco group, in data obtained in Brazil.

The estimated average heterosis ($\bar{h}_{\%}$) for the SSV was 5.3%. This value is slightly lower than that of the YLD, mainly because the $\bar{h}_{\%}$ ALK, as already mentioned, was negative, although of small magnitude. Among the best combinations for the SSV, 1x4, 3x4, 3x5, 1x5 stood out. Parental 1 and 4 were present in most cases, according to their good performance estimates per se for both traits (Table 4). The genetic difference between the inbred lines/hybrids, associated with the difference in locations, contributed to make the Genotypes x Environments interaction (GE) significant. No reports were found of the occurrence of GE with the Dark tobacco type in Brazil. However, GE has been reported in other tobacco types (Pulcinelli et al., 2014). The biggest challenge for breeders is to identify lines/hybrids with superior performance in the years following the recommendation of the cultivar(s) for the cultivation of farmers, with most of the unpredictable environmental conditions (Van Eeuwijket al., 2016). The best option, even with interaction, is to focus the results on the average of the various environments, especially because with the highest number of repetitions in obtaining the average, there is greater security in the recommendation. Thus, the emphasis on the presentation and discussion of the results are focused on the average results obtained.

Heterosis estimates with values comparable to those obtained in the present study, in tobacco from the Virginia tobacco type, were also obtained in other countries (Hancock and Lewis, 2017; Dexter-Bone and Lewis, 2019). Note that the heterosis in autogamous plants, for several characters, is lower than that observed for allogamous plants (Ramalho et al., 2012; Bernardo, 2020; Labroo et al., 2021). Furthermore, depression by inbreeding in autogamous plants is lower than that of allogamous, probably because the frequency of harmful alleles was reduced with domestication (Bernardo, 2014; Labroo et al., 2021).

The performance of a hybrid combination ($\bar{H}$) is obtained by $\bar{H}_{ij} = \bar{P} + h_{ij}$, where $\bar{P}$ is the mean of parental and h_{ij} is heterosis. For YLD, considering all hybrids, the average of the parents was responsible for 92.1% of the hybrid's performance and the hybrid (heterosis) by only 7.9%. This fact is very common in autogamous plants (Ramalho et al., 2012). In allogamous plants, such as corn, the contribution of h is much greater than the average of parental. However, it has been observed that the contribution of the average of the parental lines in relation to heterosis is increasing over time (Troyer and Wellin, 2009; Li et al., 2014).

Heterosis (h) is a function of the presence of dominance (d) in the control of characters and the divergence (y^2) between them, that is, $h = dy^2$ (Ramalho et al., 2012). Thus, for heterosis to occur there must be allelic interaction of dominance in some or all of the loci that control the character and that the parents are divergent. That is, in order to obtain good hybrids, parentals should have good average performance for the character and at the same time favorable alleles must be present in different loci, so that divergence occurs. It appears that selection should initially be directed towards obtaining good inbreed lines in all breeding programs. However, in order to obtain hybrids, different possibilities of hybrid combinations involving the best lines should be assessed at the end of the process.

Hypotheses to explain heterosis were initially proposed in the early twentieth century, as being due to allele interactions of dominance and/or over dominance. However, new hypotheses have been created forward more recently (Huang et al. 2015; Yang et al., 2017; Li et al., 2018; Andorf et al., 2019; Labroo et al., 2021). It should be emphasized that, in the case of the ALK character, both the average heterosis and the parental heterosis were not significant, showing that the dominance effects were not important for this trait, also there were no contribution, between the lines, for the heterosis. In this case, the explanation depends on the occurrence of additive x additive gene interaction (Melchinger et al., 2007; Ginkel and Ortis, 2018). This is a condition that allows heterosis to be "capitalized" even with the use of lines.

When considering the five best combinations with higher average of hybrids for the SSV estimate, only two combinations stood out in relation to the $\bar{h}_{\%}$, as previously mentioned, the combinations 3x5 and 5x9 (Table 5). The reasons for this result have already been discussed, that is, the performance of a hybrid does not depend only on the estimate of heterosis. The average of the five hybrids for YLD was 28.92 $kg.plot^{-1}$, which is higher than the average of all 45 hybrid combinations, 25.5 $kg.plot^{-1}$. For ALK, the average of the SSV from the five best combinations was practically the same average of all hybrids (Table 5).

Table 5. Means of the selection index (SSV), green leaf mass (YLD) ($kg.plot^{-1}$), and total alkaloid content (ALK) (%) of the five best and five worst hybrids selected by the SSV. Data obtained from a diallel with 10 lines of the Dark tobacco type. Assessments conducted at four locations.

	Hybrid	SSV	YLD	ALK
	1 x 4	5.76	30.63	2.87
	3 x 4	5.67	29.18	3.11
Best	3 x 5	5.66	27.52	3.42
	1 x 5	5.64	28.98	3.08
	5 x 9	5.54	28.29	3.03
	6 x 8	4.73	20.08	3.64
	6 x 7	4.72	18.94	3.88
Worst	3 x 10	4.69	23.25	2.79
	9 x 10	4.68	23.94	2.56
	2 x 6	4.66	20.22	3.43

Still considering the five best hybrids, in relation to the SSV, they stood out in relation to the YLD, is important to mention once more that the index is composed on majority by this trait. The average of the five hybrids for YLD was basically 13% above the average of all 45 hybrid combinations. The opposite occurred for ALK, the average of the SSV from the five best was 3.10%, that is, the same estimate of the average of all hybrids. The YLD average was 16.3% lower than the general average among the five worst and 5.2% higher than the general average for the ALK character. That is, the index was favorable for YLD; however, the average was lower than desired for ALK. In this case, the best option for the breeder would be to change the weights, increasing it for ALK and reducing it for YLD. Some of the selection indexes, as the SSV, can be used for flexibility to change the weight in order to fit to the objectives of the program.

Finally, an important information would be how the hybrids performed in relation to the Dark O.S. cultivar, which is the only one marketed for this tobacco type in Brazil. The averages obtained for this cultivar were YLD = 18.29 $kg.plot^{-1}$, ALK = 3.57% and SSV = 4.39. YLD was below all parental and hybrids (Table 2). Only parental 6 of ALK presented an absolute value higher than Dark O.S., the others were all inferior. This is the main reason why it remains being used by producers. However, considering the index, its behavior was below average, showing that many parental and hybrids performed better than this cultivar (Table 4). These results show that there are good options for hybrids to be used by farmers in addition to Dark O.S.

The literature contains some reports of the use of hybrids in autogamous plants, although they do not present very expressive heterosis. Tomato is a case that stands out. They use hybrids, above all, to combine lines that have a different genetic makeup for some phenotypic character expressions, with relatively simple genetic control, such as resistance to pathogens (Lippman and Zamir, 2007). In the case of results obtained in this experiment for Dark tobacco, two combinations of parental lines that had the percentage of ALK in the standard desired by the industry and consumers, and with productivity above the Dark O.S, could be chosen. One of the hybrids that would be selected by the SSV would be the 3x5 that associates YLD (48% above Dark O.S.) and a reduction of only 4.2% in the ALK (3.42%). Another combination to be suggested, for example, could be the 3x6 combination whose means were 22.69 $kg.plot^{-1}$ for YLD and 3.52% for ALK (Tables 2 and 3). The 3x6 hybrid was then 24% higher than Dark O.S. in YLD and only 1.4% lower in ALK.

This contribution of heterosis, even if of small magnitude, is of great importance for the company once hybridization is already carried out in seed production, because, for all commercial cultivars of the company, male sterility is used as a way of protecting germplasm. Therefore, it would not generate additional resource expenditure for the company in obtaining hybrids and would add, on average, 8.6% for YLD and 5.3% for the SSV.

CONCLUSIONS

The use of the sum of standardized variables selection index (SSV) allowed simultaneous gains in yield (YLD) and total alkaloid content (ALK) in Dark tobacco hybrids, although, evidently, they were of less magnitude than the selection for each characteristic alone. The average heterosis was 8.6% for YLD, -1.4% for ALK, and 5.3% for the SSV. However, considering the SSV, hybrids were obtained with an average heterosis greater than 10%. Under these conditions, the use of hybrids should be encouraged not only to combine favorable phenotypes in different parents, exploring heterosis, but also to protect the company's germplasm.

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CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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