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IDENTIFICATION OF DONOR LINES CONTAINING FAVORABLE ALLELES FOR QUANTITATIVE TRAITS IN BURLEY TOBACCO (*NICOTIANA TABACUM L.*)

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Well-adapted local lines might be used as donors to improve existing commercial hybrids. The objective of this study was to identify the best burley tobacco (*Nicotiana tabacum* L.) line among a group of four lines (SA 130, Bols 334, Barlej T and TN 90) from germplasm collection, for improving an elite single hybrid Bols 335 x TN 86. Values of three quantitative traits (number of leaves, total leaf area and leaf yield) were measured in two-year field experiments. Evaluation of donor lines as sources of new favorable alleles not present in the elite hybrid parent lines was conducted. According to Dudley (1984, 1987) the most significant class for improving quantitative traits is locus class G. Donor line TN 90

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had positive values of the parameter µG for number of leaves and for total leaf area. Studied donor lines could not be use directly as the source of new alleles for the improvement of leaf yield in elite hybrid. The improvement of leaf yield in tobacco hybrid Bols 335 x TN 86 would be conducted indirectly via back crossing (TN 86 x TN 90) x TN 86. Significance of GxE interaction for studied traits indicates the same breeding method would be effective in broad range of environmental conditions.

Key words: donor, favorable alleles, locus class, quantitative traits, tobacco, yield component

INTRODUCTION

Knowledge in gene pool of tobacco could aid in germplasm maintenance activities, provide additional information in the area of N. tabacum population genetics, and assist in selection of materials for breeding or genetic investigations (Moon et al., 2009). Tobacco breeders focus their program on those quantitative traits that are highly associated with leaf yield. Genetic bases of quantitative traits in burley and flue-cured Virginian tobacco were analyzed on different manners (DRAZIC and PRODANOVIC, 1999, DRAZIC and PRODANOVIC, 2001, DRAZIC, 2007). Some of recent studies used molecular methods to identify genetic markers that are linked to agronomically important genes (LEWIS *et al.,* 2005, 2007, MOON, 2008). However, methods of classical breeding and biometrical analyses of quantitative traits play an important and irreplaceable role in tobacco breeding (DRAZIC and SURLAN-MOMIROVIC 1990, DRAZIC 1999, DRAZIC 2001).

Period of the commercial utilization of tobacco hybrids is limited. Therefore, they must be replaced with newer high yielding hybrids. Older cultivars, lines, synthetic populations, etc. may serve for the improvement of existing tobacco hybrids, as the donors of favorable alleles. Therefore, a donor of desirable quantitative traits should be identified through the appropriate breeding programs.

The identification of favorable alleles for quantitative traits not present in elite hybrid but present in other germplasm is one of the most important tasks facing a breeder (PETROVIC *et al*., 1992. ZIVANOVIC *et al*., 2001). DUDLEY (1984, 1987) developed procedures for identifying donors containing such favorable alleles.

The questions arising in the selection of donors carrying new favorable alleles are the following:

1. Which line can be selected as the donor (P_w) if values of quantitative traits of the hybrid parental lines (P_1 and P_2) are known?

2. When donor line (P_w) is identified, should it be crossed with P_1 or P_2 of elite hybrid?

3. If donor line (P_w) is crossed with P_1, for instance, should self-fertilization start in F_2 generation, or should F_1 generation previously be crossed with P_1 or with P_w (reversible crossings) (DUDLEY, 1982)?

DUDLEY (1984, 1987) defined eight classes of loci (A–H) that existed for any three lines (P_1, P_2, P_w) on the basis of the occurrence of favorable (+) and unfavorable (-) alleles in the genotypes. If the dominant alleles are favorable, then G class loci are the most interesting, because the donor line (P_w) has plus (favorable) alleles, while P_1 and P_2 have minus (unfavorable) alleles for the trait.

The purpose of this study is to determine the best donor lines from germplasm collection of the Institute of medical plants, Belgrade, for studied quantitative traits. Results of this investigation are useful to define appropriate selection methods for improving yield and yield components in burley tobacco.

MATERIALS AND METHODS

Plant material

Four burley tobacco donor lines (SA 130, Bols 334, Berlej T i TN 90) were selected for improvement of hybrid Bols 335 x TN 86. Lines TN 86, TN 90 and SA 130 originate from the USA (MILLER, 1987), lines Bols 334 and Bols 335 from Austria (BOLSUNOV, 1961), and line Berlej T from Serbia (DRAZIC and PRODANOVIC, 2001).

Crossing design

Crossings of two parental lines with four potential donors excluding reciprocal combinations were conducted in 2000, for the purpose of producing F_1 generation seed. A comparative experiment of 6 lines and 9 hybrids (including commercial elite hybrid) was evaluated in a randomized complete block design with five repetitions in 2001 and 2002 on the experimental fields of Institute of Medical Plants, Belgrade. The area of the experimental lot was 12 m^2, and the crop density was 25000 plants/ha. Three quantitative traits (number of leaves, total leaf area and leaf yield) were measured in 10 plants per replication.

Analysis of data

Evaluation of relative values of loci in lines was conducted according to the modified model DUDLEY (1987). For two parental lines (P_1 and P_2) and donor line (P_w) there are eight locus classes (Table 1).

Table 1. Genotypes for locus classes possible for an elite hybrid (P_1 x P_2) and a potential donor line P_w (modified from DUDLEY, 1987)

Class of loci	Line 1 (P_1)	Line 2 (P_2)	Line donor (P_w)
A	+	+	+
B	+	+	-
C	+	-	+
D	+	-	-
E	-	+	+
F	-	+	-
G	-	-	+
H	-	-	-

+ = loci homozygous for favorable alleles; - = loci homozygous for unfavorable alleles;

Genotypic value of three possible genotypes (++, +-, --) on one locus are μ, aμ and -μ according to COMSTOCK and ROBINSON (1948) where the degree of dominance, μ = ½ of the difference of genotypic value ++ and -- genotypes. DUDLEY model (1987) assumes with the following: μ = constant for all loci, a = 1, full dominance, and absence of epistasis, μA = μH.

A positive value μG shows that line (P_w) has favorable alleles on loci where parental lines (P_1 and P_2) have unfavorable alleles. Value μB gives relative number of loci where P_1 and P_2 have favorable alleles, μC gives relative number of loci where P_1 and P_w have favorable alleles and P_2 does not, and μE gives to the relative number of loci where P_2 and P_w have favorable alleles and P_1 does not. Values μD and μF show that P_1 or P_2 have favorable alleles at the loci where other two lines do not have favorable alleles. In case of $a \neq 1$, (i.e., when there is no full dominance), which is one of the conditions for the use of Dudley model (1987a), the obtained values μG are not very precise. In case of $a > 1$ (over-dominance) then μG value is more precise; i.e., if $a < 1$ (partial dominance) then μG value is underestimated. The sum of μC and μF gives relative number of loci where P_1 and P_w have the same (+ or -) alleles, while μD + μE give relative number of loci where P_2 and P_w have the same (+ or -) alleles. If:

1. μC + μF > μD + μE – line P_w is more closely related to P_1 and is used for the improvement of parent P_1

2. μC + μF < μD + μE – donor line P_w is more closely related to P_2 and is used for the improvement of parent P_2

Evaluation of parentage of donor (P_w) with elite hybrid parents (P_1 and P_2) can be performed by using the following formulas $[(P_2 \text{ x } P_w) - (P_1 \text{ x } P_w) + (P_1 - P_2)/2]$. Positive value points to the parentage between P_1 and P_w, while negative value points to the parentage between P_2 and P_w (DUDLEY, 1987).

Depending on which parent is being improved, P_1 or P_2, the founding initial population for selection is determined by comparing values μD or μF with value μG. If parent P_1 is improved, there are three possibilities:

1. μD = μG; the probability that the new line will have more loci with favorable alleles in class D and G than either P_1 or P_w is maximum. Then the approach will be hybrid self-fertilization (P_1 x P_w).

2. μD > μG; points to the back crossing of hybrids (P_1 x P_w) with parent P_1.

3. μD < μG; back crossing of hybrids (P_1 x P_w) with donor P_w is recommended.

RESULTS AND DISCUSSION

Based on two-factorial variance analysis experiments with burley tobacco lines per se and single-cross hybrids, significant and highly significant means of square of genotypes, years and interactions genotypes x year were determined for all studied traits (Table 2).

In average hybrids had greater total leaf area and leaf yield than lines, which was expected (Table 3). However, hybrids had lower number of leaves than lines.

Table 2. Analysis of variance for studied quantitative traits of 9 single-cross hybrids and 6 lines evaluated in 4 replications for 2 years

Source of variation	d.f.	Mean squares of traits		
		Number of leaves	Total leaf area	Leaf yield
Genotype (G)	14	30.723**	116938.700**	1.473**
Year (Y)	1	46.875**	17017.010*	2.139**
Replication	3	4.875*	4208.253	0.005
G x Y	14	4.482*	17852.310*	0.109**
Error	87	2.007	8519.672	0.029

* = significant at 0.05 level using F-test; ** = significant at 0.01 level using F-test

Table 3. Studied quantitative traits of lines and single crosses (mean values for 2 years)

Genotype	Quantitative trait		
	Number of leaves	Total leaf area (cm^2)	Leaf yield (t/ha)
Bols 335	22.0	974.0	1.60
TN 86	26.0	664.5	1.75
SA 130 – Donor 1	27.1	682.5	1.90
Bols 334 – Donor 2	23.8	787.5	1.68
Berlej T – Donor 3	22.1	736.8	1.84
TN 90 – Donor 4	26.0	703.5	1.87
$\bar{x}$ lines	24.50	758.13	1.77
Bols 335 x TN 86	24.5	1008.5	3.24
Bols 335 x SA 130	25.1	994.9	1.67
Bols 335 x Bols 334	22.0	891.0	1.85
Bols 335 x Berlej T	19.5	831.6	1.88
Bols 335 x TN 90	24.3	994.5	2.39
TN 86 x SA 130	24.6	686.8	1.83
TN 86 x Bols 334	24.1	751.4	1.82
TN 86 x Berlej T	23.5	730.3	2.05
TN 86 x TN 90	25.3	691.1	1.77
$\bar{x}\,F_1$	23.66	842.23	2.06
$lsd_{0.05}$	0.7	94.9	0.09
$Lsd_{0.01}$	1.0	125.8	0.12

The highest number of leaves (27.1) and leaf yield (1.90 t/ha) among the lines for both years was recorded for line SA 130. Parental line Bols 335 had the highest total leaf area (974.0 cm^2).

Commercial tobacco hybrid Bols 335 x TN 86 had in average 24.5 leaves (Table 4). Parental line TN 86 had higher number of leaves (26.0) than parental line

Bols 335 (22.0). Donor lines x one of parental line hybrids had lower average number of leaves in comparison with the elite hybrid Bols 335 x TN 86.

Table 4. Average number of leaves of elite hybrid and parents of elite hybrid and their hybrids with donor lines

Bols 335	TN 86	Bols 335 x TN 86	Donor	Bols 335 x P_w	TN 86 x P_w
22.0	26.0	24.5	SA 130	25.1	24.6
			Bols 334	22.0	24.1
			Berlej T	19.5	23.5
			TN 90	24.3	25.3
			Mean	22.73	24.38

The mean comparison of parental line x donor lines P_w crosses indicates significant differences in total leaf area (Table 5). On the average, total leaf area of Bols 335 x P_w crosses (928.0 cm^2) was higher than total leaf area of TN 86 x P_w crosses (714.9 cm^2). The greatest average area of leaves was recorded for hybrid Bols 335 x SA 130 (994.9 cm^2).

Table 5. Average total leaf area (cm^2) of elite hybrid and parents of elite hybrid and their hybrids with donor lines

Bols 335	TN 86	Bols 335 x TN 86	Donor	Bols 335 x P_w	TN 86 x P_w
974.0	664.5	1008.5	SA 130	994.9	686.8
			Bols 334	891.0	751.4
			Berlej T	831.6	730.3
			TN 90	994.5	691.1
			Mean	928.0	714.9

Mean leaf yield varied from 1.67 to 2.39 t/ha in hybrids between parental lines and donors (Table 6). Elite tobacco hybrid Bols 335 x TN 86 had the highest average leaf yield (3.24 t/ha). Parental line Bols 335 had lower leaf yield (1.60 t/ha) than parental line TN 86 (1.75 t/ha), even hybrids with line Bols 335 were more productive (1.95 t/ha) than hybrids with line TN 86 (1.87 t/ha).

These differences in hybrids Bols 335 x P_w and TN 86 x P_w were expected, because certain inbred lines were assumed to be more related to one parental line than another on the basis of pedigree of lines.

For the number of leaves, it is desirable that the donor line has dominant favorable alleles which are absent from parents (P_1 and P_2) of elite hybrid. The best donor for number of leaves is TN 90 (0,750*) (Table 7). Negative value of this donor (-1.000) points to its parentage with P_2 TN 86 and indicates the best way for improvement is back crossing (P_2 x TN 90) with parent P_2 TN 86.

The highest positive values of μG for total leaf area has also TN 90 (3,156*) and therefore this line would be the best donor of favorable alleles (Table 8). Donor line TN 90 had negative value of parentage (-148,625*) suggesting greater genetic similarity with parental line P_2 TN 86. Improvement of elite hybrid Bols 335 x TN

86 for total leaf area should be conducted by improving line TN 86 via back crossing.

Table 6. Average leaf yield (t/ha) of elite hybrid and parents of elite hybrid and their hybrids with donor lines

Bols 335	TN 86	Bols 335 x TN 86	Donor	Bols 335 x P_w	TN 86 x P_w
1.60	1.75	3.24	SA 130	1.67	1.83
			Bols 334	1.85	1.82
			Berlej T	1.88	2.05
			TN 90	2.39	1.77
			Mean	1.95	1.87

Table 7. Values of parameters μG, μD and μF, parentages of donors with elite hybrid parents and the best way for founding initial population for improvement of number of leaves

Donor	μG	Parentage ($+P_1$; $-P_2$)	μD or μ F	The best way for founding initial population
SA 130	-0.219	-2.625*	-0.531*	(P_2 x D) x P_2
Bols 334	-0.094	+0.125	0.094	(P_1 x D) x P_1
Berlej T	-0.875*	+2.000*	-0.375*	(P_1 x D) x P_1
TN 90	0.750*	-1.000	0.875*	(P_2 x D) x P_2

* = larger than 2 x standard error

Table 8. Values of parameters μG, μD and μF, parentages of donors with elite hybrid parents and the best way for founding initial population for improvement of total leaf area

Donor	μG	Parentage ($+P_1$; $-P_2$)	μD or μ F	The best way for founding initial population
SA 130	2.156*	-153.375*	8.969	(P_2 x D) x P_2
Bols 334	-7.656*	+15.125*	120.906*	(P_1 x D) x P_1
Berlej T	-27.781*	+53.375*	111.344*	(P_1 x D) x P_1
TN 90	3.156*	-148.625*	10.156	(P_2 x D) x P_2

* = larger than 2 x standard error

All four lines, potential donors of favorable alleles, had negative values of parameter μG for leaf yield (Table 9). Donors SA 130 and TN 90 are more closely related to P_2 TN 86while donors Bols 334 and Berlej T are more closely related to P_1 Bols 335. Donor lines used in this study have not fixed favorable alleles at loci where elite hybrid parent lines have fixed unfavorable alleles. The improvement of leaf yield in elite hybrid Bols 335 x TN 86 should be done indirectly by improvement of yield components (number of leaves and total leaf area) via back crossing (TN 86 x TN 90) x TN 86.

Several authors used the same lines as those used in this work. Xu et al., 2004 used the tobacco line TN 86 for its transformation with the tobacco vein mottling virus (TVMV) coat protein (CP) gene. JACK *et al.,* 2008 analyzed lines TN 86 and TN 90 for alkaloids in individual plants accordingly to the LC protocol.

Method used in this study to identify the best donors for improving elite hybrid was applied in several investigations. FABRIZIUS and OPENSHAW (1994) studied new maize germplasm in relation to the target elite hybrid under improvement. DUDLEY *et al*. (1996) studied 20 maize populations as sources of favorable alleles for three elite single-cross hybrids. For grain yield, 15 of these 20 populations had significantly higher relative estimates of favorable alleles, while none of the populations proved potential for reducing ear height. DAMNJANOVIC *et al*. (2004) identified donor lines for improving fruit yield of K 35 x K 12 eggplant hybrid. MISEVIC *et al.* (1989) assumed that the well adapted local maize populations, or populations resulting from some type of family-based recurrent selection, have a satisfactory level of favorable alleles and can be used as donors for improving target genotypes. Similar results and discussion with different selection materials (lines, hybrids, populations) are given by PETROVIC and JELOVAC, 1989; PETROVIC *et al.* 1992 and DELIC, 1993. Since in previous studies researchers used different plant species, materials, different conditions of growing and production year it is hard to make direct comparisons with our results.

Table 9. Values of parameters μG, μD and μF, parentages of donors with elite hybrid parents and the best way for founding initial population for improvement of leaf yield

Donor	μG	Parentage ($+P_1$; $-P_2$)	μD or μ F	The best way for founding initial population
SA 130	-0.121	-0.915*	0.161	(P_2 x D) x P_2
Bols 334	-0.330*	-0.100	0.365*	(P_2 x D) x P_2
Berlej T	-0.238*	+0.050	0.340*	(P_1 x D) x P_1
TN 90	-0.206*	-0.695*	0.216	(P_2 x D) x P_2

* = larger than 2 x standard error

However, suggested selection method for improvement of studied tobacco hybrid could be appropriate in broad range of environmental conditions. Conclusion about improvement via back crossing was based on trait mean values calculated from experiments with significant GxE interaction.

CONCLUSION

Burley tobacco line TN 90 can be selected as the donor (P_w) of favorable alleles for improving parents of elite single cross hybrid Bols 335 x TN 86. This line has new favorable alleles for number of leaves and total leaf area not present in commercial hybrid. Based on the parentage of TN 90 with hybrid parents, the improvement for two quantitative traits should be conducted by improving line TN 86. Selected donors have not fixed dominant alleles at loci where parent lines have fixed unfavorable alleles for leaf yield. Therefore, the improvement of leaf yield in elite hybrid Bols 335 x TN 86 should be done indirectly by improvement of yield components (number of leaves and total leaf area) via back crossing (TN 86 x TN 90) x TN 86.

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IDENTIFIKACIJA LINIJA DONORA POŽELJNIH ALELA ZA KVANTITATIVNE OSOBINE BERLEJ DUVANA (*NICOTIANA TABACUM L.*)

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I z v o d

Na bazi srednjih vrednosti u dvogodišnjim ogledima, ispitivane su četiri linije (SA 130, Bols 334, Berlej 5, TN 90) iz kolekcije germplazme, kao potencijalni donori poželjnih alela za poboljšanje osobina (broj listova, površina listova i prinos lišća) elitnog prostog hibrida Bols 335 x TN 86. Analizom po metodu Dudley (1984,1987) konstatovano je da naveden linije mogu poslužiti kao potencijalni donori. Najznačajnija klasa za unapređenje kvantitativnih osobina je bila lokusna klasa G. Linija donor TN 90 ima pozitivne vrednosti parametra µG za broj listova i ukupnu površinu listova. Proučavane linije-donori, ne mogu biti korišćene direktno kao izvor novih alela za unapređenje prinosa lišća elitnog hibrida. Unapređenje prinosa kod hibrida Bols 335 x TN 86 je moguće indirektno, preko povratnog ukrštanja (TN 86 x TN 90) x TN 86. Značajnost interakcije GxE za proučavane osobine ukazuje da isti oplemenjivački metod može biti primenjen u širokom rangu ekoloških uslova.

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