

Agronomic Characteristics of Dihaploid Lines of Oriental Tobacco Obtained *in vitro*

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Summary

One of the most frequently used biotechnical methods today is the method of double haploids. By application of induced androgenesis in the *in vitro* Laboratory of Tobacco Institute - Prilep 10 dihaploid lines of oriental tobacco were obtained in 2004. For assessment of agronomic characteristics of the dihaploid lines and their analogues (P 146-7/1, Yk 301/23 and Hyb. 301/H), in the course of 2005 and 2006 three haploid lines were set up in field conditions (P 146-7/1 DH, Yk 301/23 DH and Hyb. 301/H DH) and investigations were made of the number of leaves and plant yield per hectare. With reference to the characteristic number of leaves, dihaploid lines showed a significantly low variational coefficient (CV = 1.75, 2.96: 4.70 %) compared to their analogues, which indicated that they were morphologically stable. According to their agronomic characteristic (g/plant and kg/ha), they were somewhat better or equal to those of their analogues.

Key words

oriental, tobacco, dihaploids, *in vitro*, androgenesis

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Introduction

The method of double haploids is one of the most frequently implemented biotechnical methods today. Guenter Setz (Burchet, 2004) said: "If induction of hybrids was the biggest event in selection and the use of glass houses the second biggest, technology of double haploids will be the third one".

The basic aim of this method is to reduce the breeding process in field conditions, where the process of self pollination and selection continues as long as uniformity of the varieties is achieved and until 100 % of them become homozygous. These varieties are comparable with standard varieties. It takes nine to 11 years to standardize some variety or to release a new one (Patrascu and Ioan, 1984). Namely, in reductional division of sex cells, cells with unique inherited information are developed, i.e. even in the first generations decomposition of hybrid in dihaploid lines occurs, but by doubling the genome of each haploid, the dihaploids obtained differ from their parental forms in some morphological and agronomic characteristics (Dimitrova, 1991). Yet, each dihaploid progeny is homozygous. Some authors reported that dihaploid lines achieved higher yields compared to their analogues (Zagorska et al, 1978) and the others (Arcia et al., 1978; Deaton et al., 1986) consider that dihaploid lines are less productive. Variation in yield and quality of dihaploid lines can depend greatly on the initial genotype (Deaton et al., 1982).

The aim of this paper was to study the yield characteristics of dihaploid lines of oriental tobacco, in direct creation of new tobacco varieties, and also to confirm the application of induced androgenesis depending on the aims of selection in obtaining the homozygous dihaploid lines.

Materials and methods

Estimation of dihaploid progenies in the period 2005 - 2006 in field conditions was made in Tobacco Institute - Prilep. The trial was designed as randomized block with four replications and it included three dihaploid oriental tobacco lines (P 146-7/1 DH, Yk 301/23 DH and Hyb.301/N DH) and their analogues. Results on agronomic characteristics of the investigated dihaploid lines and their analogues were statistically processed using LSD test analysis of variance (Najčeska, 2002) The main plot size was 1.87 m².

Dihaploidization was made by direct organogenesis of haploid plants, using the method of tissue culture (meristems, leaf segments 0.5 cm in size), on Murashige and Scoog (1962) nutrient medium, modified for direct organogenesis and optimized with various chemical substances: casein hydrolyzate - 1 mg/l; L-glutamine - 250 mg/l; glicine 200 mg/l; IAA-0.2 mg/l; BAP - 0.5 mg/l; adenine-20 mg/l; kinetine-3 mg/l and myoinosite-100 mg/l. The first progenies were grown in Biological laboratory in vegetative broth 3:1 (perlite:soil) up to obtaining seed material.

Results and discussion

Morphological properties

One of the most important tasks of selection is to increase the varieties yield. Knowledge of the genetic control of yield and of the factors that influence its variability in tobacco plant is essential for creation of new varieties with optimum yield (Dimitrova, 1991). By the methods of androgenesis and meristems culture (organogenesis and rhizogenesis) applied in *in vitro* laboratory of Tobacco Institute-Prilep, we obtained several dihaploid lines of oriental tobaccos 'Prilep' and 'Yaka', investigated in field conditions during 2005-2006.

Phenological investigations showed uniformity of dihaploid lines for the characteristic height of the plant with inflorescence and number of leaves per plant (Table 1).

Table 1. Morphological properties

Varieties Lines	Height of the plant with inflorescence, cm			Leaf number per plant, cm		
	0	$\delta \pm S\delta$	CV %	0	$\delta \pm S\delta$	CV %
P 146-7/1Ø	71.4	2.21±0.60	3.81	46.35	1.10±0.22	2.38
P146-7/1DH	67.05	1.33±0.30	2.14	46.65	1.21±0.27	2.57
Yk.l.301/23 Ø	122.50	5.91±1.32	5.09	50.40	3.02±0.72	5.93
Yk.l.301/23DH	126.60	5.96±1.33	4.91	47.30	1.88±0.42	3.94
Hyb.301/N Ø	74.10	1.68±0.37	2.22	35.3	0.98±0.22	2.79
Hyb.301/N DH	71.35	1.24±0.36	2.64	48.9	0.58±0.12	1.53

0 - arithmetical means (cm); δ - standard deviation; S δ - standard deviation error; CV - variational coefficient %

Investigations in 2005-2006 showed that variation of plant height was lower in dihaploid lines, compared to their analogues, and the coefficient of variation averaged from 2.14 % (P146- 7/1 DH) to 5.09 % (Yk.l 301/23 Ø). For the character leaf number per plant, all dihaploid lines also showed lower coefficient of variation compared to their analogues.

Our investigation is in agreement with those of Dimitrova (1991) and Enčeva et al. (2000) who reported lower coefficient of variation in dihaploid lines compared to their analogues, showing significant uniformity in relation to these characters.

Dry tobacco yield in dihaploid lines ranged 18.26 g/plant and 3010 kg/ha (P 146-7/1 DH and Hyb. 301/H Ø) to 21.90 g/plant and 3610 kg/ha (Yk.l. 301/23 Ø). According to this, the dihaploid line Hyb. 301/N DH achieved 14.62 % higher yield per plant and hectare compared to its analogue Hyb. 301/H Ø (18.26 g/plant; 3010 kg/ha), i.e. statistically significant difference of 1 % (Tables 2 and 3). Both dihaploid lines P 146-7/1 and Yk. l. 301/23 gave lower yields compared to their analogues.

Many authors reported that yields of dihaploid lines vary depending on whether they originate from the same haploid or from different ones (Arcia et al., 1978; Deaton et al., 1986; Šmalcelj and Čurković Perica, 2000).

Berbec and Laskowska (2003) reported that some dihaploid lines of the variety Wišlica have higher yields and some of them lower yields, compared to their analogues.

Table 2. Dry tobacco yield, g/plant

Varieties Lines	Years		Average	Difference from the control	
	2005	2006		Absolute	Relative
	P 146-7/1Ø	17.12			
P146-7/1DH	15.29	21.23	18.26	-2.82	86.62
Yk.l.301/23 Ø	19.67	24.13	21.90	-	100.00
Yk.l.301/23DH	19.32	22.54	20.93	-0.97	95.57
Hyb.301/N Ø	18.64	17.89	18.26	-	100.00
Hyb.301/N DH	21.01 ⁺⁺	20.85 ⁺⁺	20.93	+2.67	114.62
LSD 5%	1.42 ⁺⁺	2.13 ⁺			
LSD 1%	1.97 ⁺⁺	2.95 ⁺⁺			

Table 3. Dry tobacco yield, kg/ha

Varieties Lines	Years		Average	Difference from the control	
	2005	2006		Absolute	Relative
	P 146-7/1Ø	2822			
P146-7/1DH	2521	3499	3010	-465	86.62
Yk.l.301/23 Ø	3243	3978	3610	-	100.00
Yk.l.301/23DH	3185	3716	3450	-160	95.57
Hyb.301/N Ø	3072	2949	3010	-	100.00
Hyb.301/N DH	3464 ⁺⁺	3437 ⁺⁺	3450	+440	114.62
LSD 5%	86.11 ⁺	351.98 ⁺			
LSD 1%	119.30 ⁺⁺	487.65 ⁺⁺			

Table 4. Percentage of high grades (I and II), %

Varieties Lines	Years		Average	Difference from the control	
	2005	2006		Absolute	Relative
	P 146-7/1Ø	43.48			
P146-7/1DH	47.66	17.65	32.65	+2.61	108.69
Yk.l.301/23 Ø	0.00	2.16	1.08	-	100.00
Yk.l.301/23DH	5.36	5.08	5.22	+4.14	483.33
Hyb.301/N Ø	4.59	14.81	9.70	-	100.00
Hyb.301/N DH	0.00	21.54	10.77	1.07	111.03

Table 5. Average purchase price, € /kg

Varieties Lines	Years		Average	Difference from the control	
	2005	2006		Absolute	Relative
	P 146-7/1Ø	1.95			
P146-7/1DH	2.04	1.61	1.82	-0.01	99.71
Yk.l.301/23 Ø	1.69	1.48	1.58	-	100.00
Yk.l.301/23DH	1.70	1.52	1.61	+0.43	101.32
Hyb.301/N Ø	1.67	1.63	1.65	-	100.00
Hyb.301/N DH	1.63	1.67	1.65	0.00	100.00

In relation to quality, it can be stated that the investigated dihaploid lines of oriental tobacco had higher quality compared to their analogues. The higher grades percentage ranged from 1.08 % (Yk.l.301/23 Ø) to 32.65 % (P 146-7/1 DH) (Table 4). Accordingly, dihaploid lines quality was better for 8.69 % (P 146-7/1 DH), 383.33 % (Yk.l. 301/23 DH) and 11.03 % (Hyb.301/N DH), compared to their analogues.

Average purchase price varies from 1.58 €/kg for Yk l. 301/23 Ø to 1.83 €/kg for P 146-7/1 Ø. All investigated lines

Table 6. Economic effect, € /ha

Varieties Lines	Years		Average	Difference from the control	
	2005	2006		Absolute	Relative
	P 146-7/1Ø	5494.30			
P146-7/1DH	5073.20	5646.30	5359.70	-919.00	85.36
Yk.l.301/23 Ø	5496.10	5900.10	5700.80	-	100.00
Yk.l.301/23DH	5388.80	5623.50	5506.10	-194.70	96.58
Hyb.301/N Ø	5011.20	4808.50	4909.80	-	100.00
Hyb.301/N DH	5800.10 ⁺⁺	5754.00 ⁺⁺	5777.00	+867.20	117.66
LSD 5%	140.02 ⁺	577.19 ⁺			
LSD 1%	193.89 ⁺⁺	799.66 ⁺⁺			

have lower values for this parameter compared to their analogues, except for the variety Yk.l. 301/23 DH with 1.61 €/kg, which is 1.32 % higher than its analogue Yk.l. 301/23 Ø.

A part from the line Hyb.301/N DH, all dihaploid lines of investigated tobacco varieties, showed lower economic effect compared to their analogues. Economic effect of Hyb.301/N DH was 17.66 % higher compared to its analogue Hyb.301/H Ø, which is statistical difference of 1 % . This data was confirmed with the investigations of Deaton (1982), who reported that genotype from which dihaploid lines came significantly affected their yield variability and their quality level.

Conclusions

Based on the data obtained during our investigations, compared to those from the literature, the following conclusions can be drawn:

- The obtained dihaploid progenies with uniform morphological characteristics present an initial material for further investigations in breeding.
- Some of the investigated dihaploid lines (Hyb. 301/N DH) gave higher yields per stalk and per hectare for even 14.2 % compared to their analogues and they also achieved better quality.
- Biochemical methods (plant tissue culture and androgenesis) can be successfully applied in breeding programs for oriental tobaccos, in creation of new, superior homozygous lines.

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