# Development of Anther-Derived Flue-Cured Tobacco Dihaploids from PVY Resistant DH10 Hybrid

B. Šmalcelj and M. Ćurković Perica

# Entwicklung von aus Antheren stammenden „flue-cured" Tabakdihaploiden aus PVY resistenten DH10 Hybriden

## 1. Introduction

Potato virus Y (PVY) is an increasing problem affecting Croatian tobacco growers (BUŽANČIĆ, 1988). It causes significant economic losses not only on tobacco, but on all solanaceous crops. Therefore, a breeding program has been initiated to produce a flue-cured tobacco (*Nicotiana tabacum* L.) cultivar resistant to PVY, with better yield and quality than standard cultivars grown in Croatia: Drava and DH10. The source breeding material with genetic variability for resistance to PVY, yield and cured leaf quality was formed by hybridization of the Croatian line GV3 and the cv. Virginia D. The hybrid is known as the commercial cultivar DH10, which has a genetic basis for the traits of interest in this study. The parental line GV3 is resistant to blue mold caused by *Peronospora tabacina* Adam (GORNIK et al., 1973) and to PVY (SMALCELJ, 1992), but it is characterised by low quality (SMALCELJ, 1988). The German variety Virginia D is also described as resistant to PVY, but it is extremely susceptible to blue mold and black root rot caused by *Thielaviopsis basicola* Berk. and Br. Ferraris (PULULU, 1985). In comparative trials on the field of the Tobacco Institute Zagreb in Pitomača, Virginia D had a smaller growth and lower yield than the standard cultivar Drava, but it reached a higher price ($/kg) (ŠMALCELJ, 1990).

The hybrid, DH10, displays an acceptable level of resistance to PVY and blue mold, and in the Croatian growing area its yield is approximately 2 t/ha, which is better than the yield of the parental line GV3 and the cv. Virginia D. Indeed, tobacco producers have found this cultivar more acceptable than Drava since flue-curing of the leaf is relatively simple to achieve (KOZUMPLIK et al., 1992).

## Zusammenfassung

Vom „potato virus Y" (PVY) verursachte Schäden gaben Anlaß für ein Züchtungsprogramm, um die PVY-Resistenz, die Ertragsleistung und die Qualität des in Kroatien wachsenden „flue-cured" Tabak zu verbessern. Die Antheren stammen von kommerziellen DH10 Hybridpflanzen, die bereits eine Kreuzung zwischen der Linie GV3 und der Sorte Virginia D darstellen und hervorragende Eigenschaften besitzen. Aus Antheren stammende haploide Pflanzen bildeten die Grundlage für die dihaploiden Nachkommenschaften. Sie wurden aus Geweben der Mittelrippe erzeugt. In dreijährigen Feldversuchen wurden 47 Dihaploide kultiviert und deren Eigenschaften vergleichend beurteilt. Drei der 47 verwendeten Dihaploiden glichen in den agronomischen Eigenschaften dem DH10 Hybrid, übertrafen jedoch dessen den wirtschaftlichen Wert bestimmenden Qualitäten. Ihre Erträge und Resistenzeigenschaften gegenüber PVY-Viren waren vergleichbar, sie erzielten aber bessere Preise ($/kg). Diese Ergebnisse zeigen, daß haploide Tabaklinien nicht unbedingt geringere Erträge erzielen und daß die Antheren-Gewebekultur zusammen mit der Erzeugung diploider Linien eine sehr gute Methode ist, um neue, homozygote, verbesserte Tabaklinien zu erzeugen. Die vom selben Haploid stammenden Dihaploide unterscheiden sich signifikant hinsichtlich Blütezeit, Resistenz gegenüber PVY-Viren, Größe der Pflanzen, Anzahl, Breite und Länge der Blätter sowie mengenmäßigem bzw. monetärem Ertrag. Die Untersuchungen zeigen, daß sich sowohl die vom selben Haploid stammenden Dihaploide als auch die unterschiedlicher Hyploide voneinander unterscheiden können.

**Schlagworte:** Nicotiana tabacum, Dihaploide, PVY-Resistenz, Ertrag, Qualität.

**Summary**

Damage caused by potato virus Y (PVY) has motivated a breeding program which aimed at the production of a flue-cured tobacco cultivar resistant to PVY, with better yield and quality than standard cultivars grown in Croatia. Anthers were collected from the commercially grown hybrid DH10, a cross of line GV3 and cv. Virginia D, with favourable agronomic traits. Dihaploides were raised from the midvein explants of anther-derived haploids. Forty-seven dihaploids raised in this experiment were evaluated in comparative field trials, over a 3-year period. Three out of 47 tested dihaploid lines equaled agronomic and exceeded commercial properties of DH10 hybrid. Their yield and PVY-resistance were similar to that of the DH10 hybrid, but the price ($/kg) was considerably higher. These results show that dihaploid tobacco lines do not necessarily exhibit yield reduction and that anther culture followed by diploidization is a useful method to accelerate the development of new, improved, homozygous tobacco varieties. Significant differences between dihaploids derived from the same haploid plant were observed in flowering date, PVY resistance, plant height, number of leaves, leaves width and length, yield and price, showing that dihaploid lines derived from the same haploid individual can differ among themselves as well as those derived from different haploids.

**Key words:** *Nicotiana tabacum*, dihaploids, PVY resistance, yield, quality

The objective of the present breeding program was to use anther culture to accelerate the development of completely homozygous varieties bearing the desirable traits of the DH10 hybrid. By comparing them to their parents and commercially grown varieties, dihaploid lines raised in this experiment were evaluated for their agronomic and commercial traits as well as for resistance to PVY.

## 2. Materials and Methods

Anther culture of tobacco hybrid DH10 was established following the procedure of NITSCH and NITSCH (1969). Buds were collected from plants grown in the field and treated at 4° C for 24 h. Anthers were sterilised in 70 % ethanol (10 s) and in 1.5 % Izosan-G (a chlorine based product purchased from Pliva, Zagreb) (5 min) and placed on the medium recommended by BAJAJ (1983). Haploid plantlets generated *via* anther culture were transferred to a medium described by KASPERBAUER and WILSON (1979). Midvein explants from mature haploids were cultured to generate dihaploid shoots on the medium recommended by KASPERBAUER and COLLINS (1972), supplemented with 2 mg/l kinetine. Separated shoots were transferred to MS medium (MURASHIGE and SKOOG, 1962) supplemented with organic constituents according to KASPERBAUER and WILSON (1979). Midvein-derived plants were grown to maturity and self-pollinated.

Seeds were collected from 47 dihaploids ($2n = 4x = 48$) derived from 29 haploids ($n = 2x = 24$). In this way, some dihaploids were derived from the same haploid individual. Haploidy of the anther-derived plants was generally verified by the lack of seed production, but the ploidy level of those plants as well as of those regenerated after diploidization was also cytologicaly analysed as described by ĆURKOVIĆ PERICA et al. (1997). Each haploid raised in anther culture was indicated by a number which was than used combined to a letter, to designate the dihaploid, thus indicating from which haploid it was derived.

Dihaploid lines were tested in field comparative tests in Pitomača during 1995, 1996 and 1997. A randomized complete-block design with four replications was used. Entries were planted in single-row plots containing 20 plants each. Only selected dihaploid lines that displayed at least one desirable trait were retested again in the following year. The cultivars Drava, DH10, Virginia D and the line GV3 were used as standards. The variety McNair 944 was used as a standard for the evaluation of PVY occurrence. Symptoms of PVY infection, as described by LUCAS (1975), were visually examined. Susceptibility to PVY was expressed as the number of plants (0–80) displaying the symptoms according to ŠMALCELJ (1989). Holes (Ø up to 5 cm) in leaf tissue that developed from small injuries caused according to PLESS and MILLER (1986) by plant bags in the early stage of leaf growth, were observed in the populations of some dihaploids and expressed as a number in the range 0 to 80. In the comparative field tests where no plant bag injuries were observed, this parameter was omitted from the results. Yield, price ($/kg), pre-flowering time, the number of bag-injured plants and PVY symptoms were determined in all field tests, while last leaf height, number of leaves and $10^{th}$ leaf length and width were only measured in comparative field tests 2 and 4.

Data for all traits were analyzed by ANOVA within each year. An LSD value was calculated for comparison between entries.

## 3. Results

The first anther-generated plantlets appeared after 4 weeks of cultivation. Cytological analyses showed that they were all haploid. Midvein cultures of 346 haploid individuals produced 389 regenerants. Only 12% of the regenerated plants were dihaploid and some of those dihaploids were raised from the same haploid plant. Finally, 47 dihaploids derived from 29 haploids could be evaluated in field tests.

In comparative field test 1 (1995) eight dihaploid lines were tested (Table 1). Although lines 3-A and 3-B are dihaploids derived from the same haploid progenitor, they significantly differed in susceptibility to PVY. Line 3-A was resistant to PVY and its yield and price equaled those of standards. The agronomic performance of this line was confirmed in a repeated test in 1996. (Table 5). All other dihaploid lines evaluated in this field test performed worse than the standards for at least one trait.

Comparative field test 2 (1995) was almost devastated by PVY (Table 2). All plants of the susceptible variety McNair 944 displayed symptoms of PVY infection and in the standard cultivars Drava and DH10 one fourth of the plants showed symptoms. In such condition line 207-B showed desirable resistance to PVY. Unfortunately, repeated test did not confirm the superior performance of this line (Table 5). Lines 215-A and 215-K had neither agronomic nor commercial value (Table 2).

In comparative field test 3 (1995) dihaploid 228-L was resistant to PVY and reached high price (Table 3), but because of the low yield it has only a breeding value. Dihaploid lines 236-B and 239-K showed an adequate resistance to PVY and reached higher price than standards, but some of their chemical traits are not desirable in flue-cured tobacco (data not shown).

None of the lines tested in comparative field test 4 (Table 4) had a sufficient commercial value. Nine dihaploid lines tested in comparative field test 5 (1995) performed worse than line GV3 (data not presented).

In comparative field test 2 (1995) (Table 2), differences were observed among dihaploid lines derived from the same haploid. Line 207-C performed poorer than line 207-B for all traits. Significant differences[1] in price(**) and pre-flowering period(**) were also observed between dihaploid lines 238-B and 238-C, as well as between lines 239-A and 239-K, and 240-K and 240-L, respectively (Table 3). Characteristic plant bag injuries were observed in progenies 240-K and 240-L, and in 5 dihaploid lines derived from haploid 244 (Table 4), so that all dihaploids derived from the same haploid exhibited this trait. On the other hand differences were observed between 244-B and 244-D in plant height 13 cm (**) length 5 cm (**) and width 3.9 cm (**) of the tenth leaf.

Table 1: Comparative field test 1: Agronomic and commercial traits of 8 dihaploid lines and control tobacco cultivars, grown in the field during 1995
Tabelle 1: Vergleichender Fehlversuch 1: Pflanzenbauliche und ökonomische Eigenschaften 8 dihaploider Tabaklinien und von Kontrollsorten unter Feldbedingungen 1995

| Cultivar | Plants with bag induced injuries 0-80 | Plants with PVY symptoms 0-80 | Pre-flowering period No. of days | Yield t/ha | Price $/kg |
|---|---|---|---|---|---|
| DH 10 | 0 | 1 | 66 | 2,0 | 1,31 |
| Drava | 0 | 1 | 62 | 2,2 | 1,12 |
| McNair 944 | 0 | 41 | 74 | 1,6 | 1,12 |
| Virginia D | 0 | 2 | 78 | 1,9 | 1,17 |
| GV3 | 0 | 5 | 62 | 1,9 | 1,11 |
| 3-A | 0 | 0 | 70 | 2,1 | 1,11 |
| 3-B | 0 | 6 | 67 | 2,0 | 1,02 |
| 21-A | 0 | 1 | 67 | 2,1 | 0,86 |
| 23-E | 0 | 1 | 78 | 1,5 | 1,03 |
| 36-B | 0 | 16 | 61 | 1,5 | 1,40 |
| 37-B | 0 | 5 | 62 | 2,0 | 1,32 |
| 42-D | 80 | 7 | 63 | 1,7 | 1,17 |
| 44-A | 0 | 10 | 64 | 1,5 | 0,85 |
| LSD 5% | - | - | 5 | 0,3 | 0,15 |
| LSD 1% | - | - | 7 | 0,4 | 0,22 |

Table 2: Comparative field test 2: Agronomic and commercial traits of 9 dihaploid lines and control tobacco cultivars, grown in the field during 1995
Tabelle 2: Vergleichender Feldversuch 2: Pflanzenbauliche und ökonomische Eigenschaften 9 dihaploider Tabaklinien und von Kontrollsorten unter Feldbedingungen 1995

| Cultivar | Plants with PVY symptoms 0-80 | Pre-flowering period No. of days | Yield t/ha | Price $/kg | Last leaf height cm | No. of leaves | Tenth leaf length cm | Tenth leaf width cm |
|---|---|---|---|---|---|---|---|---|
| DH 10 | 21 | 65 | 2,0 | 1,26 | 120 | 16 | 42 | 21 |
| Drava | 25 | 62 | 2,0 | 1,09 | 120 | 17 | 39 | 16 |
| McNair 944 | 80 | 69 | 1,1 | 0,89 | 90 | 20 | 38 | 17 |
| Virginia D | 27 | 71 | 2,0 | 1,23 | 120 | 21 | 46 | 25 |
| GV3 | 33 | 63 | 1,8 | 0,97 | 110 | 15 | 43 | 19 |
| 201-B | 14 | 66 | 1,6 | 1,17 | 110 | 16 | 39 | 18 |
| 207-A | 17 | 70 | 1,9 | 1,14 | 130 | 19 | 42 | 21 |
| 207-B | 8 | 67 | 2,0 | 1,29 | 150 | 18 | 41 | 20 |
| 207-C | 31 | 77 | 1,3 | 1,09 | 100 | 18 | 39 | 18 |
| 207-D | 24 | 68 | 1,8 | 1,12 | 130 | 16 | 42 | 21 |
| 207-E | 28 | 66 | 1,8 | 1,14 | 130 | 21 | 44 | 20 |
| 207-F | 21 | 66 | 1,9 | 1,11 | 130 | 19 | 43 | 21 |
| 215-A | 72 | 58 | 1,0 | 0,86 | 100 | 16 | 39 | 16 |
| 215-K | 61 | 55 | 1,1 | 0,94 | 90 | 16 | 38 | 17 |
| LSD 5% | - | - | 0,4 | 0,15 | 20 | 3 | 5 | 2 |
| LSD 1% | - | - | 0,5 | 0,20 | 30 | 4 | 7 | 3 |

Table 3: Comparative field test 3: Agronomic and commercial traits of 9 dihaploid lines and control tobacco cultivars, grown in the field during 1995
Tabelle 3: Vergleichender Feldversuch 3: Pflanzenbauliche und ökonomische Eigenschaften 9 dihaploider Tabaklinien und von Kontrollsorten unter Feldbedingungen 1995

| Cultivar | Plants with bag induced injuries 0-80 | Plants with PVY symptoms 0-80 | Pre-flowering period No. of days | Yield t/ha | Price $/kg |
|---|---|---|---|---|---|
| DH 10 | 0 | 0 | 63 | 2,2 | 1,08 |
| Drava | 0 | 0 | 59 | 2,3 | 1,05 |
| McNair 944 | 0 | 44 | 66 | 2,2 | 1,29 |
| Virginia D | 0 | 1 | 66 | 2,4 | 1,42 |
| GV3 | 0 | 7 | 59 | 2,4 | 0,85 |
| 228-L | 0 | 0 | 73 | 1,7 | 1,46 |
| 236-B | 0 | 0 | 71 | 1,8 | 1,19 |
| 237-K | 0 | 0 | 67 | 1,9 | 1,15 |
| 238-B | 0 | 0 | 56 | 1,9 | 0,66 |
| 238-C | 0 | 0 | 68 | 1,6 | 1,09 |
| 239-A | 0 | 8 | 56 | 1,6 | 1,00 |
| 239-K | 0 | 0 | 73 | 1,8 | 1,23 |
| 240-K | 80 | 1 | 71 | 1,8 | 0,99 |
| 240-L | 80 | 0 | 64 | 1,6 | 1,09 |
| LSD 5% | - | - | 6 | 0,5 | 0,15 |
| LSD 1% | - | - | 8 | 0,7 | 0,20 |

In comparative field test 11 (1996) lines 289-A, 289-B, 289-C performed similarly or better than the parents (Table 5). Their estimated price was higher than that of the better standard and they were resistant to PVY. They were not significantly different among themselves for any presented trait. Agronomic and commercial traits of those three lines were confirmed in a repeated field test in 1997 (Table 6).

## 4. Discussion

Problems with reduced yield in cultured dihaploid tobacco lines have been observed by many researchers (ARCIA et al., 1978; BURK and CHAPLIN, 1980; BURK and MATZINGER, 1976; COLLINS et al., 1974; DEATON et al., 1986). Therefore, these lines are usually considered to be of breeding

Table 4: Comparative field test 4: Agronomic and commercial traits of 6 dihaploid lines and control tobacco cultivars, grown in the field during 1995
Tabelle 4: Vergleichender Feldversuch 4: Pflanzenbauliche und ökonomische Eigenschaften 6 dihaploider Tabaklinien und von Kontrollsorten unter Feldbedingungen 1995

| Cultivar | Plants with bag induced injuries 0-80 | Plants with PVY symptoms 0-80 | Pre-flowering period No. of days | Yield t/ha | Price $/kg | Last leaf height cm | No. of leaves | Tenth leaf length cm | Tenth leaf width cm |
|---|---|---|---|---|---|---|---|---|---|
| DH 10 | 0 | 0 | 60 | 2,2 | 1,20 | 114 | 17,9 | 41 | 20,1 |
| Drava | 0 | 2 | 59 | 2,3 | 1,02 | 118 | 19,8 | 42 | 18,1 |
| McNair944 | 0 | 58 | 64 | 2,2 | 1,14 | 98 | 21,2 | 42 | 18,1 |
| Virginia D | 3 | 4 | 68 | 2,4 | 1,48 | 112 | 19,7 | 43 | 22,5 |
| GV3 | 0 | 1 | 58 | 2,1 | 0,89 | 120 | 19,4 | 42 | 19,3 |
| 244-A | 80 | 0 | 64 | 2,0 | 0,82 | 96 | 18,1 | 38 | 19,1 |
| 244-B | 80 | 0 | 68 | 2,1 | 0,82 | 103 | 18,0 | 42 | 21,6 |
| 244-C | 80 | 0 | 65 | 2,0 | 0,69 | 96 | 18,2 | 39 | 18,9 |
| 244-D | 80 | 3 | 65 | 1,8 | 0,75 | 90 | 17,7 | 37 | 17,7 |
| 244-L | 80 | 1 | 64 | 2,1 | 0,77 | 94 | 18,3 | 37 | 17,7 |
| 246-A | 0 | 0 | 64 | 1,8 | 1,09 | 115 | 18,8 | 44 | 19,5 |
| LSD 5% | - | - | 4 | 0,3 | 0,12 | 8 | 1,7 | 4 | 1 |
| LSD 1% | - | - | 5 | 0,4 | 0,22 | 10 | 2,2 | 5 | 1,3 |

Table 5: Comparative field test 11: Agronomic and commercial traits of 9 dihaploid lines and control tobacco cultivars, grown in the field during 1996
Tabelle 5: Vergleichender Feldversuch 11: Pflanzenbauliche und ökonomische Eigenschaften 9 dihaploider Tabaklinien und von Kontrollsorten unter Feldbedingungen 1996

| Cultivar | Plants with PVY symptoms 0-80 | Pre-flowering period No. of days | Yield t/ha | Price $/kg |
|---|---|---|---|---|
| DH 10 | 3 | 71 | 1,9 | 1,17 |
| Drava | 0 | 70 | 2,4 | 1,03 |
| McNair 944 | 46 | 75 | 1,6 | 1,11 |
| Virginia D | 2 | 78 | 1,3 | 1,11 |
| GV3 | 0 | 70 | 2,0 | 0,97 |
| 3-A | 0 | 73 | 1,9 | 1,09 |
| 207-B | 0 | 76 | 1,8 | 1,00 |
| 228-L | 1 | 80 | 1,1 | 1,17 |
| 295-A | 3 | 75 | 1,6 | 0,77 |
| 296-A | 3 | 70 | 1,8 | 0,99 |
| 296-B | 1 | 72 | 1,7 | 0,92 |
| 298-A | 0 | 74 | 2,0 | 1,25 |
| 298-B | 0 | 72 | 1,9 | 1,35 |
| 298-C | 0 | 77 | 1,9 | 1,34 |
| LSD 5% | - | 5 | 0,3 | 0,12 |
| LSD 1% | - | 6 | 0,4 | 0,17 |

value only (SCHNELL and WERNSMAN, 1986). Nevertheless, BURK and CHAPLIN (1980), WALKER and AYCOCK (1994), SMALCELJ (1996) and MENCHEY and AYCOCK (1998) suggested that exceptional individuals can occure in dihaploid populations derived from a hybrid anther source. In our field tests, certain dihaploid lines performed similarly or at a higher level than both parents for agronomic and commercial traits and displayed resistance to PVY. This result shows that dihaploid lines with high quality and yield can be developed through anther culture, thus shortening the time needed in conventional procedures to produce new, superior, homozygous tobacco cultivars.

Table 6: Comparative field test 13: Agronomic and commercial traits of 3 dihaploid lines and control tobacco cultivars, grown in the field during 1997
Tabelle 6: Vergleichender Feldversuch 13: Pflanzenbauliche und ökonomische Eigenschaften 3 dihaploider Tabaklinien und von Kontrollsorten unter Feldbedingungen 1997

| Cultivar | Plants with PVY symptoms 0-80 | Pre-flowering period No. of days | Yield t/ha | Price $/kg |
|---|---|---|---|---|
| DH10 | 1 | 64 | 2,1 | 1,35 |
| Drava | 3 | 65 | 2,4 | 1,31 |
| McNair944 | 11 | 69 | 2,3 | 1,51 |
| GV3 | 2 | 66 | 1,9 | 1,29 |
| Virginia D | 0 | 71 | 1,8 | 1,46 |
| 298-A | 1 | 66 | 2,6 | 1,54 |
| 298-B | 2 | 68 | 2,1 | 1,54 |
| 298-C | 0 | 63 | 2,3 | 1,54 |
| LSD 5% | - | 5 | 0,4 | 0,14 |
| LSD 1% | - | 6 | 0,5 | 0,19 |

Among dihaploid lines derived from the same haploid individual differences were observed in yield, price, plant height, number of the leaves, length and width of the tenth leaf, pre-flowering period and susceptibility to PVY. Although theoretically all dihaploid lines derived from the same haploid should display identical or at least similar traits, mutagenic effects of the dihaploid technique (ARCIA et al., 1978) as well as amplification of DNA sequences during formation of dihaploids (DHILLON et al., 1983; REED et al., 1992; REED and WERNSMAN, 1989) could have caused the differences between those lines. The only trait that was common for all dihaploids raised from the same haploid was susceptibility to plant bags.

These results show, for the first time, that dihaploid lines albeit derived from the same haploid can perform at different levels for a number of traits and should therefore be tested as well as lines derived from different haploids. Therefore, dihaploids derived from the same haploid deserve researchers' and breeders' attention as well as those derived from the different haploids.

## References


ARCIA, M. A., E. A. WERNSMAN and L. G. BURK (1978): Performance of anther-derived dihaploids and their conventionally inbred parents as lines, in $F_1$ hybrids, and in $F_2$ generations. Crop Sci. 18, 413–418.

BAJAJ, Y. P. S. (1983): In vitro production of haploids. In: EVANS, D. A., W. R. SHARP, P. V. AMMIRATO and Y. YAMADA (Eds.): Handbook of Plant Cell Culture, vol. I; Techniques for propagation and breeding. MacMillan publishing CO., New York, 228–287.

BURK, L. G. and J. F. CHAPLIN (1980): Variation among anther-derived haploids from a multiple disease-resistant tobacco hybrid. Crop Sci. 20, 334–338.

BURK, L. G. and D. F. MATZINGER (1976): Variation among anther-derived doubled haploids from an inbred line of tobacco. J. Hered. 67, 381–384.

BUŽANČIĆ, A. (1988): Occuring of necrotic strain of potato virus Y in flue-cured tobacco in Yugoslavia. Tutun/Tobacco 38 (5–6), 175–190 (in Croatian with English summary).

COLLINS, G. B., P. D. LEGG and C. C. LITTON (1974): The use of anther-derived dihaploids in Nicotiana, II: Comparison of doubled haploid lines with lines obtained by conventional breeding methods. Tob. Sci. 18, 40–42.

ĆURKOVIĆ PERICA, M., F. GILLET, A. JACQUIN-DUBREUIL, M. KRSNIK-RASOL and S. JELASKA (1997): Nicotine content in transformed haploid and dihaploid tissues of tobacco (*Nicotiana tabacum* L.). Phyton 37, 229–239.

DEATON, W. R., G. B. COLLINS and M. T. NIELSEN (1986): Vigor and variation expressed by anther-derived doubled haploids of burley tobacco (*Nicotiana tabacum* L.), I: Comparison of sexual and doubled-haploid populations. Euphytica 35, 33–40.

DHILLON, S. S., E. A. WERNSMAN and J. P. MIKSCHE (1983): Evaluation of nuclear DNA content and heterokromatin changes in anther-derived dihaploids of tobacco *(Nicotiana tabacum)* cv. Coker 139. Can. J. Genet. Cytol. 25, 169–173.

GORNIK, R., K. DEVČIĆ and P. BUKOVAC (1973): New hybrid varieties of Virginia from Tobacco Institute Zagreb. Tutun/Tobacco 1–12, 17–23 (in Croatian with English summary).

KASPERBAUER, M. J. and G. B. COLLINS (1972): Reconstitution of diploids from leaf tissue of anther-derived haploids in tobacco. Crop Sci. 12, 98–101.

KASPERBAUER, M. J. and H. M. WILSON (1979): Haploid production and use. In: DURBIN, R. D. (Ed.): Nicotiana; Procedures for Experimental Use. U.S. Department of Agriculture, Technical Bull, 33–39.

KOZUMPLIK, V., N. VULETIĆ and F. PRPIĆ (1992): Influence of mineral fertilization, locality and season on certain characteristics of DH10 – new flue-cured tobacco variety. Agronomski glasnik 3, 185–193 (in Croatian with English summary).

LUCAS, G. B. (1975): Vein banding. In: LUCAS, G. B. (ed.): Diseases of Tobacco, Harold E. Parker and Sons, Printers, Fuquay-Varina, N.C., 457–477.

MENCHEY, E. K. and M. K. AYCOCK (1998): Anther-derived dihaploids for lodging improvement in tobacco. Crop Sci. 38, 698–701.

MURASHIGE, T. and F. SKOOG (1962): A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol. Plant. 15, 473–497.

NITSCH, J. P. and C. NITSCH (1969): Haploid plants from pollen grains. Science 163, 85–87.

PLESS, G. D. and R. D. MILLER (1986): Injury by the tarnished plant bag to selected potyvirus-resistant burley tobacco lines. Tob.Sci. 30, 127–129.

PULULU, M. N. (1985): Contribution à l'étude de l'utilisation des triazoles dans la lutte contre la pourriture noir des racines (Chalara elegans) du tabac. Ann.du Tabac SEITA 219, 5–71.

REED, S. M., J. A. BURNS and E. A. WERNSMAN (1992): Cytological comparison of amplified chromosome segments in four tobacco doubled haploids. Crop Sci. 32, 362–366.

REED, S. M. and E. A. WERNSMAN (1989): DNA amplification among anther-derived doubled haploid lines of tobacco and its relationship to agronomic performance. Crop Sci. 29, 1072–1076.

SCHNELL, R. J. and E. A. WERNSMAN (1986): Androgenic somaclonal variation in tobacco and estimation of its value as a source of novel genetic variability. Crop Sci. 26, 84–88.

ŠMALCELJ, B. (1988): Chemical composition of the flue-cured tobacco variety Drava and line GV3. Agronomski glasnik 5, 19–24 (in Croatian with English summary).

ŠMALCELJ, B. (1989): Observation of different tolerances of some flue-cured genotypes to PVY and TMV in the conditions of natural infection in the field. CORESTA Meet. Agro.-Phyto Group, Cesme, 1989, CORESTA Inform. Bull. 4, 48–69.

ŠMALCELJ, B. (1990): Some characteristics of flue-cured tobacco variety Virginia D. Agronomski glasnik 1–2, 37–42 (in Croatian with English summary).

ŠMALCELJ, B. (1992): GV3 line resistance to PVY evaluated in the conditions of natural infection in the field. Tutun/Tobacco 42, 115–118 (in Croatian with English summary).

ŠMALCELJ, B. (1996): Some characteristics of flue-cured tobacco hybrid GV3 x NC567 pollen dihaploids. Agronomski glasnik 1, 3–14 (in Croatian with English summary).

WALKER, D. R. and M. K. AYCOCK (1994): Development of anther-derived dihaploids to combine disease resistance in Maryland tobacco. Crop Sci. 34, 335–338.

## Notes

[1] *,** significant at P = 0.05 and 0.01, respectively

## Address of authors

**Dr. Blažica Šmalcelj**, Tobacco Institute Zagreb, Planinska 1, 10000 Zagreb, Croatia.

**Dr. Mirna Ćurković Perica***, University of Zagreb, Faculty of Science, Dept. of Biology, Marulićev trg 20/II, 10000 Zagreb, Croatia; e-mail: mirna@croatica.botanic.hr

* corresponding author

Eingelangt am 12. Mai 1999

Angenommen am 5. November 1999