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# Embryogenic responsibility of *Brassica oleracea* vegetables in a microspore culture

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**ABSTRACT**: Sixteen commercial cultivars and landraces of white and red cabbage, six cultivars and self-pollinated lines of kohlrabi, one commercial and three experimental $F_1$ hybrids of cauliflower and two lines of Savoy cabbage were tested for pollen embryogenesis. Microspore cultures were conducted by the procedure which provided the best results in majority of previously tested *Brassica oleracea* genotypes. Microspore division was induced in all tested vegetable species but the embryo development often stopped after several days in the proembryo stage. The high frequency of embryogenesis was achieved in one commercial open-pollinated cabbage cv. Holt and two cabbage landraces as well as in all cauliflower accessions tested. The first generation of crosses between previously derived doubled haploid (DH) regenerants of $R_1$ generation and until now non-responsive commercial cultivars of cauliflower proved to be highly embryogenic. Formerly obtained $R_1$ regenerants of cauliflower derived from five hybrid cultivars were tested in the glasshouse and investigated for the ploidy level, fertility and curd quality. Two $R_2$ DH lines of kohlrabi and one line of Brussels sprouts were evaluated in field trials and they appeared to be promising for further breeding. Some regenerants proved to be self-sterile due to reduced development of anthers though the number of chromosomes in pollen mother cells was found normal.

**Keywords**: *Brassica oleracea;* microspore culture; embryogenic responsibility; doubled haploid

Regeneration of doubled haploid plants from microspores could be a useful tool for acceleration of homozygous line production in breeding programmes of *Brassica* crops. The successful haploid embryo production in microspore cultures was reported in most *Brassicaceae* species (LICHTER 1989; DUIJS et al. 1992). *Brassica oleracea* L. includes several remarkably different morphological forms, such as broccoli, Brussels sprouts, cabbage, cauliflower, kale and kohlrabi. These forms also differ in their ability to produce haploid regenerants (RUDOLF et al. 1999). The problem of practical application of the microspore culture technique is a very low embryo yield in many of cole crop genotypes (CARLOS, DIAS 1999). Sufficient production of microspore derived plants in a wide range of genotypes is a prerequisite for the use of a doubled haploid system for rapid introduction of specific traits in *Brassica* vegetable breeding.

Our previous work (VYVADILOVÁ et al. 1998a,b) was focused on improvement of the microspore culture technique and investigation of factors affecting embryogenesis in a microspore culture of selected *Brassica oleracea* vegetables. Statistical analysis did not prove a significant effect of any level of individual factors. However it can be concluded that the main factors affecting microspore embryogenesis are genotype specificity and developmental stage of microspores.

The aim of the study presented in this paper was to test the embryogenic response of microspore cultures in a broader spectrum of *Brassica oleracea* genotypes from *Brassica* germplasm collection maintained in Gene Bank of RICP, Olomouc workplace, and some breeding materials. A possibility to improve the responsibility of non-responsive cauliflower cultivars by means of crossing with highly responsive doubled haploids was investigated as well. The previously optimized microspore culture procedure which proved successful in majority of the cole crop genotypes was used on a larger scale. The doubled haploid lines will be evaluated for agronomic and quality traits with an emphasis on fungal disease and virus resistance. Promising lines will be used in breeding programmes.

## MATERIAL AND METHODS

Experiments were conducted on sixteen head cabbage (*convar. capitata* (L.) Alef. *var. alba* DC and *var. rubra* DC.) cultivars and landraces; five kohlrabi (*var. gongylodes* L.) open-pollinated cultivars and one self-pollinated line; two Savoy cabbage (*convar. capitata* (L.) Alef. *var. sabauda* L.) lines, one commercial $F_1$ hybrid and three experimental $F_1$ hybrids from the crosses of doubled haploid (DH) regenerants $R_1$ × non-responsive commercial cultivars of cauliflower (*var. botrytis* L.). Donor

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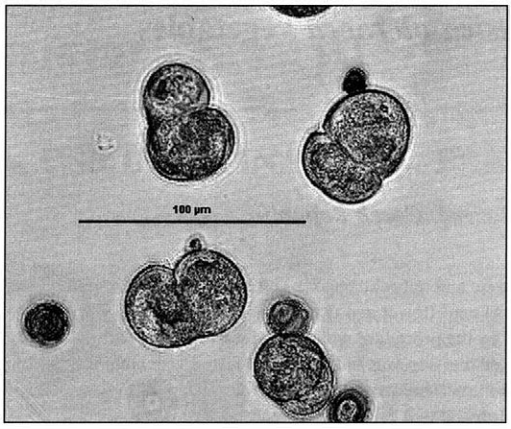


Fig. 1. First microspore division (cabbage cv. Holt)

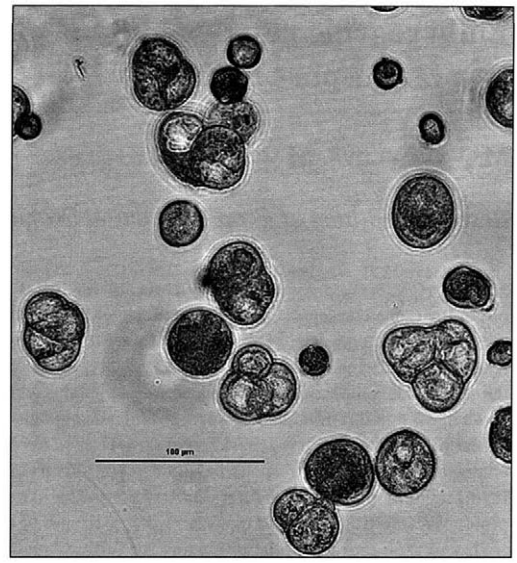


Fig. 2. Multicellular structures (cabbage cv. Holt)

plants of cauliflower cv. Siria $F_1$ were grown in a glasshouse and $R_1$ DH Plana $F_1$ × Brilant, $R_1$ DH Kashmere $F_1$ × Brilant and $R_1$ DH Plana $F_1$ × Fortuna in outdoor isolation cages during June. After vernalization for 4 months at 4°C in a cold room, all accessions of cabbage, kohlrabi and Savoy cabbage were grown in the growth chamber under controlled environmental conditions with 16 h photoperiod and day/night temperature 12/18°C.

Microspores from late uninucleate to early binucleate developmental stages were isolated for cultivation in liquid NLN medium (LICHTER 1985). The microspore cultures were incubated for 24 h at 35°C and further cultured at 26°C in darkness till the stage of developed embryogenic structures. Then they were transferred onto a gyratory shaker (60 rpm) at 16/8 photoperiod (VYVADILOVÁ et al. 1998a). For each accession one to three 100 ml Petri dishes and minimum two replicates were performed. The process of embryogenesis in individual genotypes and the number of regenerated embryos were evaluated.

Recently obtained $R_1$ regenerants from five cauliflower hybrid cultivars, two $R_2$ DH lines of kohlrabi and one line of Brussels sprouts were evaluated for the ploidy level, fertility and quality parameters in a glasshouse and field trials.

## RESULTS AND DISCUSSION

Most of the selected vegetable genotypes proved to be responsible in a microspore culture, but development of green cotyledonary embryos was sufficiently successful only in some of them (Figs. 3, 4). Microspore enlargement was induced in all tested vegetable species at the beginning of the culture but sometimes the cell division

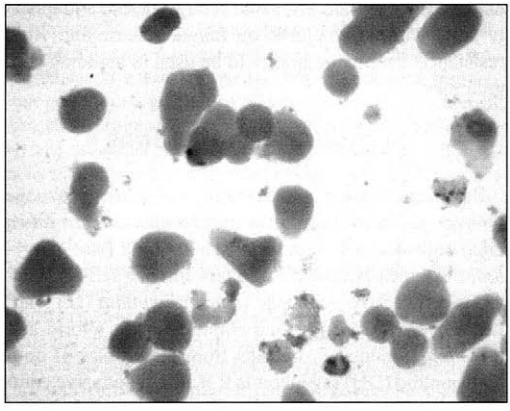

Fig. 3. Embryos in early developmental stages (length max. 2 mm, cabbage Landrace-Lutiše)

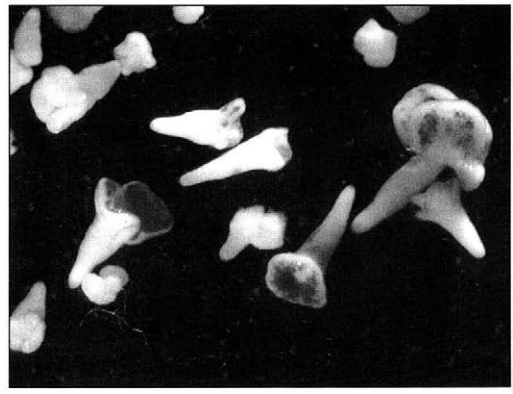

Fig. 4. Cotyledonary embryos of DH Plana $F_1$ × cv. Fortuna (max. length 3 mm)

Table 1. Isolated microspore culture responsiveness of some *Brassica oleracea* vegetable genotypes

| *Brassica* vegetable | Cultivar | Bud length (mm) | Microspore division | Embryos in early developmental stages | Green cotyledonary embryos |
|---|---|---|---|---|---|
| Cabbage | Dauerweiss | 6.5 – 7.0 | + | + | + |
| | Pourovo pozdní | 6.5 – 7.0 | + | – | – |
| | Holt | 6.0 – 6.5 | +++ | +++ | +++ |
| | Vysocké horské červené | 6.5 – 7.0 | – | – | – |
| | Klokotské | 6.5 – 7.0 | – | – | – |
| | Křimické 1 | 4.5 – 5.5 | + | + | + |
| | Křimické 2 | 5.0 – 5.5 | + | + | + |
| | Landrace (Lutiše) | 5.5 – 6.0 | +++ | +++ | +++ |
| | Landrace (Lutiše 46) | 6.0 – 6.5 | + | + | + |
| | Landrace (Zakamenné) | 7.0 – 7.5 | + | + | + |
| | Landrace (Zázrivá 367) | 5.5 – 6.0 | +++ | +++ | +++ |
| | Polar | 6.5 – 7.0 | + | – | – |
| | Pourovo červené | 6.0 – 6.5 | – | – | – |
| | Pourovo polopozdní | 5.5 – 6.0 | – | – | – |
| | Táborské | 6.5 – 7.0 | – | – | – |
| | Trvanlivé D | 6.5 – 7.0 | ++ | ++ | ++ |
| Cauliflower | $R_1$ DH Kashmere $F_1$ × Brilant | 5.5 – 6.0 | +++ | +++ | +++ |
| | $R_1$ DH Plana $F_1$ × Brilant | 5.0 – 5.5 | +++ | +++ | +++ |
| | $R_1$ DH Plana $F_1$ × Fortuna | 5.0 – 5.5 | +++ | +++ | +++ |
| | Siria $F_1$ | 4.5 – 5.5 | +++ | +++ | +++ |
| Kohlrabi | Domino | 6.5 – 7.5 | + | + | + |
| | Luna | 5.5 – 6.0 | + | + | + |
| | Matouškova modrá raná | 5.5 – 6.0 | + | + | + |
| | Szentesi Nyari Feher | 5.5 – 6.0 | – | – | – |
| | Szentesi Ozsi Feher | 5.5 – 6.0 | + | + | + |
| | Line P7 | 6.0 – 7.0 | + | + | + |
| Savoy cabbage | Line Cidlina | 6.5 – 7.5 | – | – | – |
| | Line Orlice | 7.0 – 7.5 | + | + | + |

\+ – positive reaction

++ – more than 50 embryos per Petri dish

+++ – high responsibility – more than 100 embryos per Petri dish

(Figs. 1, 2) did not proceed or it stopped within eight days or in the proembryo stage. Some accessions, especially cabbage cultivars, formed abnormal misshapen embryos (Fig. 5).

The high frequency of embryogenesis was achieved in one commercial open-pollinated cabbage cv. Holt and two cabbage landraces. The first generation of crosses between previously derived doubled haploid regenerants $R_1$ ($R_1$ DH ) and non-responsive commercial cultivars of cauliflower as well as the commercial $F_1$ hybrid Siria also proved to be highly embryogenic. Enhancement of embryo yield by crossing of recalcitrant genotype with responsible DH regenerant was reported by RUDOLF et al. (1999) in white cabbage. Very low efficiency of androgenic embryo production was observed in the tested genotypes of Savoy cabbage and kohlrabi (Table 1).

Two formerly obtained $R_2$ DH lines of kohlrabi, two lines of cauliflower and one line of Brussels sprouts appeared to be promising for further breeding. But many of

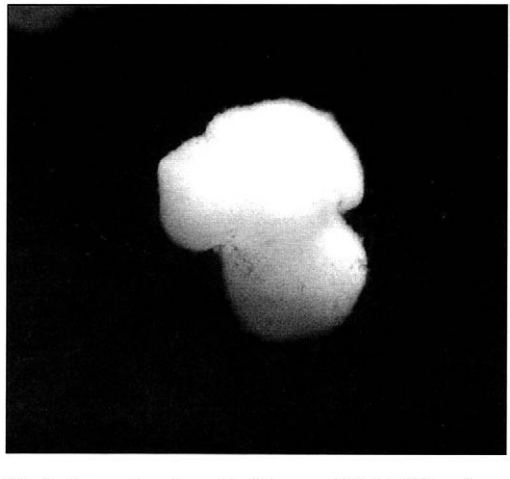

Fig. 5. Abnormal embryo of cabbage cv. Křimické (2 mm)

the doubled haploid regenerants proved to be self-sterile due to reduced development of anthers though the number of chromosomes in pollen mother cells was found normal. This phenomenon was also reported by STIPIC and CAMPION (1997) in cauliflower. It is necessary to study the cause of male sterility that could complicate the use of the doubled haploid method in breeding programmes.

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## Embryogenní responzibilita zelenin druhu *Brassica oleracea* v mikrosporové kultuře


**ABSTRAKT**: Šestnáct komerčních a krajových odrůd bílého a červeného hlávkového zelí, šest odrůd a samoopylených linií kedlubnu, jedna hybridní odrůda a tři experimentální $F_1$ hybridy květáku a dvě linie hlávkové kapusty byly testovány na embryogenní schopnost. Pro mikrosporové kultury byl zvolen postup, kterým bylo dosaženo nejlepších výsledků u většiny dříve testovaných genotypů *Brassica oleracea*. Dělení mikrospor bylo indukováno u všech zeleninových druhů, ale vývoj embryí se často zastavil po několika dnech ve stadiu proembryí. Vysoké frekvence embryogeneze bylo dosaženo u odrůdy Holt a dvou krajových odrůd hlávkového zelí a u všech testovaných genotypů květáku. Jako vysoce embryogenní se projevila $F_1$ generace kříženců dříve odvozených dihaploidních (DH) regenerantů a dosud neresponzibilních odrůd květáku. Dříve získané $R_1$ regeneranty květáku odvozené od pěti hybridních odrůd byly testovány ve skleníku z hlediska stupně ploidie, fertility a kvality růžice. Na základě hodnocení $R_2$ generace v polních pokusech byly vybrány dvě DH linie kedlubnu a jedna linie růžičkové kapusty perspektivní pro další šlechtění. Některé regeneranty se vyznačovaly samčí sterilitou v důsledku redukovaného vývoje prašníků, přestože byl zjištěn diploidní počet chromozomů v pylových mateřských buňkách.



**Klíčová slova**: *Brassica oleracea*; mikrosporová kultura; embryogenní responzibilita; dihaploidy



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# Heritability estimation of some fruit traits of apricots

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**ABSTRACT**: One qualitative and five quantitative fruit traits were studied in parent varieties Hargrand and C4R8T22 and their progeny in 1998 and 1999. The crossing was made as a part of the apricot-breeding program at the Faculty of Horticulture in Lednice. Fruit firmness, fruit weight, stone separability from flesh and stone weight were measured and the percentage proportion of flesh weight in the whole fruit weight (flesh quotient) was calculated. All trait values were measured immediately after fruit harvest. The main aim of the paper was to estimate the heritability of the above-mentioned quantitative traits. The kernel flavour was observed as a qualitative trait with Mendelian inheritance. High heritability was estimated in both years only for stone separability. The estimation of heritability for other quantitative traits significantly differed each year. The bitter kernel flavour was determined as a recessive Mendelian trait.

**Keywords**: apricots; quantitative traits; heritability; fruit traits

The genetic improvement in fruit tree species obtained by means of traditional breeding methods requires a large area and a long time, all the more as the knowledge of inheritance mechanisms in these species is generally poor. Better knowledge of inheritance in these species would allow researchers to save both time and space.

The fruit tree traits very often show continuous variations, requiring the use of quantitative genetic analyses. Both empirical experience and experimental results of breeders make us draw a conclusion that most traits in apricots are quantitatively inherited.

In spite of the lack of information about genetic determination of the important traits in apricots, plant breeders have some extensive empirical experience. Vavilov probably started the first apricot-breeding program in the twenties of the last century in Russia. About fifty years ago the apricot breeding started in Canada, USA and other countries with suitable conditions for apricot growing (BAILEY, HOUGH 1975). During the 1960s, the first experimental and breeding orchards were established in Lednice. The main aims of apricot breeding programs in Lednice have been formulated as follows:

– regular and high fruit production through a decrease in its variability (variability caused by abiotic and biotic unfavourable factors),

– high quality of fruits both for direct consumption and for processing,

– enlargement of the harvest period by breeding cultivars with different ripening period (VACHŮN et al. 1986).

Better knowledge of apricot inheritance and of important traits heritability would be a valuable asset to breeders by facilitating the parent choice and would allow them to achieve the formulated aims faster and more cheaply. However, such knowledge is still relatively poor in this species.

Heritability has been studied for instance on different *Prunus* species such as cherry (HANSCHE et al. 1966), peach (HANSCHE et al. 1972) and almond (DICENTA et al. 1993). In apricots the correlation of progenies with parents for four fruit traits was established by LAPINS et al. (1957). On the other hand, most authors only simply indicate the distribution of progenies by a range of values, or the percentage of individuals showing a higher level of the observed traits (PAUNOVIČ, PLAZINIČ 1976; PAUNOVIČ 1987). Some authors sometimes observed partial dominant effects in apricot progenies (PAUNOVIČ, MIŠIČ 1975; KOSTINA 1977). The values of the parents used for crossing in breeding programs were also estimated (KOSTINA, ZAGORODNAJA 1975; KOSTINA 1977). Most detailed analysis of inheritance in apricots was made by COURANJOU (1995), who estimated heritability of eleven quantitative traits by studying six parents and their progenies in half-diallel crossing.

This paper examines the progeny and parents from the apricot-breeding program of Faculty of Horticulture in Lednice with respect to estimations of heritability values of several fruit traits.

## MATERIAL AND METHODS

### Locality characteristics

All data for genetic analyses were collected in a breeding orchard in Lednice in 1998 and 1999. Lednice lies in the south-easten part of the Czech Republic with a warm and dry climate. Its altitude is 164 m, average annual precipitation is 524 mm, precipitation during the vegetation period is 323 mm (61% of the annual sum), and average annual temperature is 9°C. The winter season is usually mild with only several short frost periods.

### Parents and progeny

The cultivar Hargrand was used as a maternal parental component and C4R8T22 as a paternal component. The crossing was made in 1990 and the seedlings were planted in a breeding orchard at a spacing 6 × 1 m in spring 1992. Pruning of trees was minimal to shorten the time period before the beginning of fruit production. In the years 1998 and 1999 altogether 60 trees were evaluated for the purpose of this paper. The progeny was labelled H.540.

The cultivar Hargrand was bred by K. O. Lapins and Catherine H. Bailey at Canadian Agriculture Research Station Summerland, British Columbia in 1966. It possesses intermediate growth, good frost resistance, large fruits and it is partly resistant to *Xanthomonas pruni* and *Monilinia laxa* and tolerant to *Leucostoma* spp. (LAYNE 1981).

C4R8T22 is an interesting hybrid from New Jersey, USA. Its phenotype is similar to Chinese cultivars. It has a very long dormant period (about 50 days). The fruits are large with very bad stone separability (GÓTH 1996).

### Traits studied

All observations were made on fresh harvested fruits. Fruits were harvested in the same maturity stage and therefore different harvest dates were used for the different trees of progeny and parents. The harvest dates are indicated in Table 1.

Table 1. The dates of fruit harvests

| Year | C4R8T22 | Hargrand | Progeny H.540 |
|---|---|---|---|
| 1998 | July, 6th | July, 22nd | July, 7th, 8th, 9th, 12th, 13th |
| 1999 | July, 10th | August, 2nd | July, 10th, 11th, 12th |

Altogether 30 fruits from each parent variety and 15 fruits from each tree of progeny were examined.

The weight of the whole fruit was determined immediately after harvest. Flesh firmness was measured with a hand penetrometer FT 327 and the results were given in MPa. The separability of stone from flesh was evaluated on a nine-point scale (1 – non-separable, 3 – badly separable, 5 – medium separable, 7 – well separable, 9 – excellently separable) and the separated stone was weighed. The percentage of flesh weight in the whole fruit weight (flesh quotient) was calculated.

The kernel taste was determined as a subjective trait by tasting. Three types of kernel taste were expected: bitter, slightly bitter and sweet.

### Statistical analysis

All data were processed by Microsoft Excel 97 and by Unistat 4.53 for Excel. Initially, a two-way analysis of variance (ANOVA) was carried out where the genotype and environment were tested as sources of variability. Consequently, the significance of differences between the trees was determined by confidence intervals.

However, the parent trees were vegetatively propagated each from one source, their variance was used as an environmental variance ($s^2_E$) estimation. Variance of the whole progeny was used as a phenotypic variance ($s^2_P$). Genotypic variance ($s^2_G$) was calculated as a difference between phenotypic and environmental variance. Heritability ($h^2$) was estimated as a quotient of genotypic ($s^2_G$) and phenotypic ($s^2_P$) variances, so called broad-sense heritability (LUSH 1941):

$$h^2 = \frac{s^2_G}{s^2_P}$$

## RESULTS AND DISCUSSION

The results of the two-way ANOVA, where genotype end environment were determined as sources of variability, are summarized in Table 2. It is clear from the table that except for stone separability, the variability of all other traits was strongly influenced by the environment.

Only the values of stone separability are distributed asymmetrically in the progeny while the values of all

II. Results of two way ANOVA for quantitative characters

| Character | Source of variability | *F* - value | | Significance |
|---|---|---|---|---|
| | | calculated | critical | |
| Fruit weight | Genotype | 1.78 | 1.53 | * |
| | Environment | 34.60 | 7.07 | ** |
| Stone weight | Genotype | 2.10 | 1.83 | ** |
| | Environment | 22.32 | 7.07 | ** |
| Flesh quotient | Genotype | 1.65 | 1.53 | * |
| | Environment | 15.58 | 7.07 | ** |
| Flesh firmness | Genotype | 0.87 | 1.53 | n.s. |
| | Environment | 122.25 | 7.07 | ** |
| Stone separability | Genotype | 4.52 | 1.83 | ** |
| | Environment | 2.95 | 4.00 | n.s. |

* significant at the level $\alpha = 0.05$, ** significant at the level $\alpha = 0.01$, n.s. – not significant

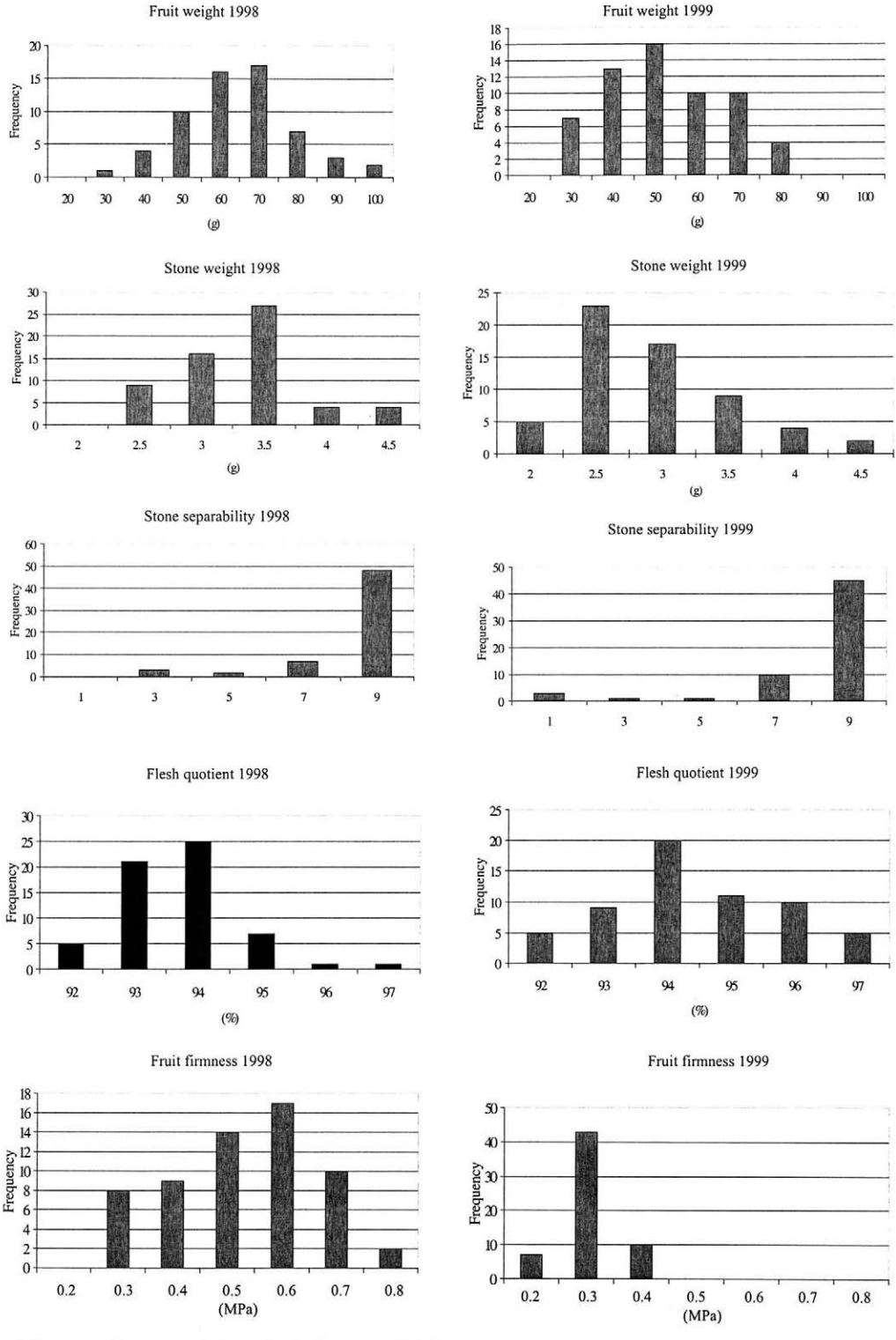


Fig. 1. Frequency histograms of observed traits in progeny H.540

Table 3. Estimation of heritability for fruit traits in progeny H.540

| Trait | Year | Variance | | | Heritability |
|---|---|---|---|---|---|
| | | phenotype | environment | genotype | |
| Fruit weight | 1998 | 217.2 | 262.7 | 0.0 | 0.0# |
| | 1999 | 202.7 | 68.6 | 134.1 | 0.7 |
| Stone weight | 1998 | 0.3 | 0.3 | 0.0 | 0.0# |
| | 1999 | 0.3 | 0.2 | 0.1 | 0.4 |
| Flesh quotient | 1998 | 0.9 | 0.9 | 0.0 | 0.0# |
| | 1999 | 1.8 | 1.1 | 0.7 | 0.4 |
| Flesh firmness | 1998 | 0.020 | 0.002 | 0.018 | 0.9 |
| | 1999 | 0.002 | 0.01 | 0.0 | 0.0# |
| Stone separability | 1998 | 2.2 | 0.0 | 2.2 | 1.0 |
| | 1999 | 4.0 | 1.9 | 2.1 | 0.5 |

# whole variability was caused by the environment

other traits display normal symmetric variability (Fig. 1). It probably resulted from the observed facts that only heritability of stone separability was possible to estimate in both years as it is shown in Table 3. Consequently, it follows that good stone separability seems to be a very good inherited trait with partly dominant inheritance. Similar results were published by KRŠKA (1996) and by PAUNOVIČ (1987). The fruits of parental cultivars Hargrand and C4R8T22 and their stone separability are presented in Figs. 2 and 3 respectively.

Heritability of all other traits could be calculated only in one of the two years of observation. In the second one, the parent variance could not be used as an estimation of environmental variance because of its high value. In spite of that in 1999, very high heritability was estimated for fruit weight, which correlates with experiments of COURANJOU (1995). On the other hand, lower values of heritability for fruit traits in peaches were published by DE SCORSA et al. (1998).

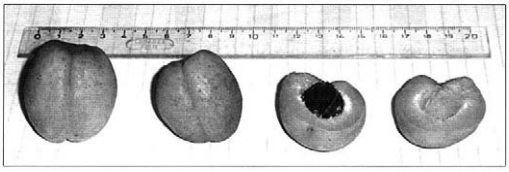

Fig. 2. Fruits and stone separability of parent cultivar Hargrand

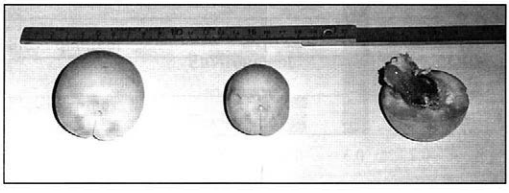

Fig. 3. Fruits and stone separability of parent cultivar C4R8T22

We also examined the taste of kernel in the framework of our analysis. The results were classified into three categories: bitter, slightly bitter and sweet. However, only bitter taste was found in both parents as well as in the whole progeny. These results suggest the monogenic inheritance for kernel taste with bitter taste as a recessive trait. KOSTINA (1969) and KRŠKA (1996) drew the same conclusions. On the other hand, BASSI and NEGRI (1991) found about 40% individuals with sweet kernel in the progeny of two parents having a bitter kernel.

The main aim of this paper was to estimate the heritability of some fruit traits in apricot. It may be used to predict the potential genetic improvement to be found in the progeny derived from parents selected for their phenotypic traits. There are several ways how to calculate heritability value. We calculated it as a proportion of the total phenotypic variance for which genetic differences are responsible. The so called broad-sense heritability (LUSH 1941; RIEGER et al. 1976) as well as our experimental data were suitable for this calculation. However, heritability values calculated in this way strongly depend on a good estimation of the environmental portion of the variation, which was estimated as a parental variation. This is a weak point of the used method. However, the high heritability value of stone separability was found in both years.

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## Odhad heritability některých plodových znaků meruněk

**ABSTRAKT**: V letech 1998 a 1999 bylo sledováno pět kvantitativních a jeden kvalitativní znak u rodičovských odrůd Hargrand a C4R8T22 a jejich potomstva. Křížení bylo uskutečněno jako součást šlechtitelského programu meruněk na Zahradnické fakultě MZLU v Lednici. Pevnost dužniny, hmotnost plodu, odlučitelnost pecky a hmotnost pecky byly přímo měřeny a následně byl dopočítán procentuální podíl dužniny na celkové hmotnosti plodu. Hodnoty uvedených znaků byly měřeny bezprostředně po sklizni plodů. Hlavním cílem práce byl odhad heritability jednotlivých studovaných znaků. Jako kvalitativní znak s mendelistickou dědičností byla sledována také chuť jádra. Vysoká hodnota heritability byla v obou letech zjištěna pouze u odlučitelnosti pecky. U ostatních znaků se odhad heritabilit v jednotlivých letech výrazně lišil. Hořká chuť jádra se projevila jako recesivní mendelistický znak.

**Klíčová slova**: meruňky; kvantitativní znaky; dědivost; plodové znaky

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# Tree vigour of new apple cultivars grown in the Czech Republic and some factors influencing it

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**ABSTRACT**: Vigour of apple trees on M 9 rootstock was monitored in 35 orchards located in different climatic conditions of the Czech Republic between 1996–2000. Altogether 31 cultivars, commonly grown or perspective for the country, were ranged according to their synthetic growth index based on the increase of trunk-cross-section area, canopy volume, and mean shoot length. Cultivars with the least tree vigour (Braeburn and Delor) grew more than 50% weaker than the most vigorous ones (Bohemia and Rubín). A large part of the differences between cultivars in tree vigour was related to differences in their yields. Another important factor that influenced tree vigour in this study was the density of planting. Climatic conditions were also a factor of great influence on the vigour of apple-trees. In warmer regions of lower altitudes, trees were generally more vigorous than those growing in "cold" conditions of higher altitudes. A response of some cultivars to climatic conditions was somewhat different from the others.

**Keywords**: apple; cultivars; tree vigour; climatic conditions; tree spacing; M 9 rootstock; productivity

Vegetative growth of fruit trees is a complex process and direct methods of its estimation have not as yet been developed. It can be determined indirectly on the basis of the trunk thickness or trunk circumference, the number of annual shoots and their total length (OSTROWSKA, CHELPINSKI 1997).

Tree vigour in the case of apple cultivars is a very important characteristic, because it determines the choice of a growing system, a rootstock, and tree spacing. Knowledge of the characteristic is relevant for growers especially in the case of new cultivars, with which hitherto long-term experience from their own growing practices is absent. In the majority of pomological books, including those issued recently, the vigour of cultivars is classified very roughly. Usually only three categories of vigour are mentioned: weak, medium and vigorous (MORGAN, RICHARDS 1993; FISCHER 1995). Also, the descriptions of new cultivars are described in the same manner (WAY 1979; GRASLUND 1989; RUEß 2000).

In the Czech Republic, a complex research of tree vigour of apple cultivars in different climatic and growing conditions was rendered twenty years ago (PAPRŠTEIN et al. 1983). Since that time in this country, the system of growing has essentially changed, and the development of growing a series of new cultivars has emerged. A relatively great number of new perspective cultivars are currently recommended to growers in the country (BLAŽEK 1994, 1995).

The purpose hereof was to study and evaluate tree vigour among the most frequently planted and perspective cultivars in different climatic conditions in the Czech Republic. At the same time, the researchers wanted to determine which factors in this country mostly influence tree vigour in contemporary growing conditions.

## MATERIAL AND METHODS

Within two subsequent research projects funded by the Ministry of Agriculture of the Czech Republic between 1996–2000, altogether 35 apple orchards located in different climatic conditions in the country were monitored for the performance of new cultivars. From every region of the Czech Republic, the best enterprises were chosen for this study as well as producers who were more or less engaged in testing the new cultivars. For the purpose of this study, data concerning growth parameters of trees and their productivity from orchards on M 9 rootstock were used. The most typical form in the orchards were the slender spindles planted in single rows with planting distances corresponding to densities of approximately 2,500 trees per hectare. The age of the majority of the trees frequently ranged from two to seven years.

From every cultivar in the orchard, usually six randomly selected trees were measured and evaluated. Upon these, the measurement of average trunk-cross-section area (TCA), canopy volume (CV) and shoot length were calculated. Yield per tree was estimated by counting fruits and the number was multiplied by their average weight, which was obtained by weighing a small sample. Climatic conditions of growing area were classified into

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three groups based on average annual mean temperature: cold (below 7.5°C), medium (7.5–9°C) and warm (above 9°C). These categories are more or less linked to conditions in the Czech Republic with decreasing altitude: over 400 m, 200–400 m, and less than 200 m above sea level respectively.

For the final classification of cultivars according to tree vigour, a synthetic growth index was used. This index was constructed by the sum of trunk-cross-section area, canopy volume and shoot length, conveyed to the percentage of the total mean. Cultivars in which the number of replications was equal or higher than 5 were only included into the final tables. On this basis, altogether 31 cultivars were studied. All results were processed by ANOVA and regression analysis.

## RESULTS

The largest differences in tree vigour of cultivars and between different climatic areas were found in a comparison on the basis of trunk cross-section area (Table 1). The highest mean year's increase of TCA (4.27 $cm^2$) was observed in Bohemia, a solid red mutant of the original variety Rubín, which practically had the same level of vigour (4.15 $cm^2$). On the contrary, the lowest mean year's increase of TCA was observed in Braeburn – 1.84 $cm^2$. In percentage, it is approximately 56% smaller than in the case of Bohemia.

On average, trees classified according to TCA were the most vigorous in warm climatic areas whereas their growth in cold climatic areas was the weakest – the TCA

Table 1. Effect of cultivars and climatic area on year's increase of TCA ($cm^2$)

| Cultivar | Climatic area | | | Mean |
|---|---|---|---|---|
| | cold | medium | warm | |
| Angold | 2.13 | 2.40 | 2.77 | 2.43 |
| Bohemia | 3.72 | 4.19 | 4.90 | 4.27 |
| Braeburn | 1.64 | 1.52 | 2.37 | 1.84 |
| Delor | 1.83 | 2.05 | 2.18 | 2.02 |
| Elstar | 2.42 | 2.92 | 3.70 | 3.01 |
| Florina | 2.80 | 3.30 | 3.69 | 3.26 |
| Gala | 2.37 | 2.70 | 3.32 | 2.79 |
| Gloster | 3.10 | 3.20 | 3.47 | 3.26 |
| Golden Delicious | 2.27 | 2.55 | 3.16 | 2.66 |
| Goldstar | 2.54 | 2.72 | 3.14 | 2.80 |
| Granny Smith | 2.51 | – | 4.05 | 3.28 |
| Idared | 2.42 | 2.76 | 3.31 | 2.83 |
| Jarka | 2.09 | 2.34 | 2.64 | 2.36 |
| Jonagold | 2.84 | 3.25 | 4.33 | 3.47 |
| Jonalord | 2.07 | 2.21 | 2.28 | 2.19 |
| Julia | 2.34 | 3.07 | 3.59 | 3.00 |
| Karmína | 2.03 | 2.33 | 2.83 | 2.40 |
| Melodie | 2.15 | 2.56 | 3.41 | 2.71 |
| Melrose | 3.01 | 3.67 | 4.28 | 3.66 |
| Otava | 1.80 | 2.28 | 2.46 | 2.18 |
| Produkta | 1.83 | 1.95 | 2.42 | 2.07 |
| Rajka | 2.28 | 2.56 | 3.31 | 2.72 |
| Resista | 2.29 | 2.71 | 3.50 | 2.83 |
| Rosana | 2.10 | 1.89 | 2.92 | 2.30 |
| Rubín | 3.75 | 3.99 | 4.71 | 4.15 |
| Rubinola | 3.58 | 4.06 | 4.74 | 4.13 |
| Selena | 1.87 | 2.27 | 2.44 | 2.19 |
| Šampion | 3.03 | 3.45 | 3.44 | 3.31 |
| Topaz | 2.39 | 2.99 | 3.26 | 2.88 |
| Vanda | 2.08 | 1.98 | 3.02 | 2.36 |
| Zuzana | 2.16 | 2.41 | 2.82 | 2.46 |
| Total | 2.43 | 2.74 | 3.30 | 2.83 |
| $LSD_{0.05}$ | 0.37 | 0.39 | 0.48 | – |

Table 2. Effect of cultivars and climatic area on year's increase of canopy volume ($m^3$)

| Cultivar | Climatic area | | | Mean |
|---|---|---|---|---|
| | cold | medium | warm | |
| Angold | 0.71 | 0.81 | 0.87 | 0.84 |
| Bohemia | 1.37 | 1.45 | 1.68 | 1.50 |
| Braeburn | 0.49 | 0.73 | 0.83 | 0.68 |
| Delor | 0.54 | 0.76 | 0.71 | 0.67 |
| Elstar | 0.80 | 0.93 | 1.16 | 0.86 |
| Florina | 0.98 | 1.19 | 1.25 | 1.14 |
| Gala | 0.87 | 0.93 | 1.02 | 0.94 |
| Gloster | 0.96 | 1.16 | 1.22 | 1.11 |
| Golden Delicious | 0.76 | 0.83 | 1.14 | 0.91 |
| Goldstar | 0.69 | 0.79 | 1.07 | 0.85 |
| Granny Smith | 0.98 | – | 1.30 | 1.14 |
| Idared | 0.77 | 0.87 | 1.02 | 0.89 |
| Jarka | 0.68 | 0.79 | 0.84 | 0.77 |
| Jonagold | 0.94 | 1.06 | 1.22 | 1.07 |
| Jonalord | 0.51 | 0.76 | 0.81 | 0.69 |
| Julia | 0.73 | 0.79 | 0.98 | 0.83 |
| Karmína | 0.58 | 0.73 | 0.92 | 0.75 |
| Melodie | 0.63 | 0.89 | 1.06 | 0.86 |
| Melrose | 1.12 | 1.29 | 1.33 | 1.24 |
| Otava | 0.66 | 0.80 | 0.98 | 0.82 |
| Produkta | 0.76 | 0.80 | 1.06 | 0.87 |
| Rajka | 0.83 | 0.90 | 1.05 | 0.93 |
| Resista | 0.88 | 0.97 | 1.20 | 1.02 |
| Rosana | 0.62 | 0.63 | 0.88 | 0.71 |
| Rubín | 1.27 | 1.48 | 1.57 | 1.44 |
| Rubinola | 1.36 | 1.37 | 1.47 | 1.40 |
| Selena | 0.79 | 0.83 | 0.94 | 0.86 |
| Šampion | 0.73 | 0.84 | 0.99 | 0.85 |
| Topaz | 0.72 | 0.91 | 1.01 | 0.88 |
| Vanda | 0.60 | 0.56 | 0.98 | 0.72 |
| Zuzana | 0.70 | 0.81 | 0.95 | 0.82 |
| Total | 0.81 | 0.92 | 1.08 | 0.94 |
| $LSD_{0.05}$ | 0.16 | 0.19 | 0.15 | |

value was about 26% smaller on average than in warm areas. However, the reaction to climatic conditions varied among particular cultivars. The greatest reduction of vigour in cold climatic conditions was observed in the case of Granny Smith – nearly 38%. A more distinct reduction of vigour in cold areas was found in Melodie, Julia, Elstar, Resista, Janagold, Rajka, Vanda, Braeburn and Melrose. On the contrary, with Jonalord, Gloster and Šampion the reduction of vigour in cold areas was negligible.

Bohemia was also the most vigorous cultivar according to the year's increase of canopy volume (Table 2). Its mean increase of canopy volume was equal to 1.5 $m^3$, whilst with Delor which had the smallest canopy, the value reached only 0.67 $m^3$. On the average, trees in medium areas had canopy volume of approximately 15% and in cold areas 25% smaller than trees in warm areas. The greatest reduction of vigour in cold areas was observed in the cases of Braeburn, Melodie and Vanda, whilst the reductions with Rubinola, Gala, Melrose and Selena were the smallest.

For the final evaluation of cultivar vigour, a synthetic growth index is used (Table 3). All cultivars, which were included in this study, were ranged starting with the cultivar of the weakest vigour – Braeburn to the most vigorous one – Bohemia. The vigour of Braeburn, in terms of the synthetic growth index, was equal to half that of Bohemia. Among the group of cultivars possessing weak vigour, the list included Braeburn, as well as Delor, Vanda, Rosana, Jonalord and Karmína. The following culti-

Table 3. Cultivars arranged to increasing tree vigour expressed by synthetic growth index (%)

| Cultivar | Components of growth index (% of the mean) | | | Synthetic growth index |
|---|---|---|---|---|
| | mean increase of TCA | mean increase of canopy volume | mean length of shoots | |
| Braeburn | 65.2 | 72.9 | 73.0 | 211.1 |
| Delor | 71.4 | 71.7 | 77.7 | 220.8 |
| Vanda | 83.4 | 76.5 | 71.4 | 231.3 |
| Rosana | 81.4 | 75.8 | 79.6 | 236.9 |
| Jonalord | 77.4 | 74.0 | 87.4 | 238.9 |
| Karmína | 84.9 | 79.6 | 83.7 | 248.2 |
| Otava | 77.1 | 87.1 | 92.0 | 256.2 |
| Jarka | 83.4 | 82.5 | 90.5 | 256.4 |
| Zuzana | 87.0 | 87.7 | 89.9 | 264.7 |
| Julia | 106.1 | 88.5 | 71.3 | 265.9 |
| Produkta | 73.1 | 93.0 | 105.7 | 271.8 |
| Topaz | 101.9 | 94.2 | 82.3 | 278.4 |
| Angold | 86.0 | 89.3 | 105.3 | 280.6 |
| Selena | 77.6 | 91.4 | 113.3 | 282.3 |
| Melodie | 95.7 | 92.1 | 96.6 | 284.3 |
| Goldstar | 99.0 | 90.7 | 95.0 | 284.7 |
| Rajka | 96.1 | 99.2 | 92.0 | 287.3 |
| Šampion | 116.9 | 90.9 | 85.1 | 293.0 |
| Golden Delicious | 94.2 | 97.2 | 102.1 | 293.5 |
| Elstar | 106.6 | 92.2 | 101.2 | 300.0 |
| Idared | 100.2 | 94.6 | 105.9 | 300.7 |
| Gala | 98.8 | 100.3 | 103.7 | 302.9 |
| Resista | 100.1 | 108.8 | 123.5 | 332.4 |
| Gloster | 115.2 | 118.6 | 111.4 | 345.2 |
| Jonagold | 122.8 | 114.4 | 113.0 | 350.2 |
| Granny Smith | 116.1 | 121.6 | 115.3 | 353.0 |
| Florina | 115.5 | 121.8 | 135.5 | 372.7 |
| Melrose | 129.3 | 132.7 | 119.5 | 381.5 |
| Rubinola | 145.9 | 148.9 | 129.3 | 424.1 |
| Rubín | 146.9 | 153.5 | 125.9 | 426.3 |
| Bohemia | 151.1 | 159.8 | 121.9 | 432.8 |

vars were classified with below average vigour: Otava, Jarka, Zuzana, Julia, Produkta and Topaz. The medium vigorous cultivars were: Angold, Selena, Melodie, Goldstar, Rajka, Šampion, Golden Delicious, Elstar, Idared and Gala. Among the cultivars with above average vigour, there were Resista, Gloster, Jonagold and Granny Smith. As vigorous, there were Florina and Melrose. Finally as very vigorous, there were Rubinola, Rubín and Bohemia.

Tree vigour was also significantly influenced by productivity. Negative regressions were found between specific yield and increase of both trunk cross-section area and canopy volume (Figs. 1 and 2). With high productivity or overcropping, the vigour of trees was distinctly reduced. This relationship obviously influenced classification of vigour in a range of cultivars. The most vigorous cultivars – Bohemia, Rubín and Rubinola were less productive and on the contrary the most productive ones – Produkta, Jonalord and Otava had moderate or weak vigour (Table 4). Interrelations between lower productivity and greater tree vigour is also evidently responsible in some cultivars for smaller differences in vigour between cold and warm regions.

The last factor, which influenced vigour of trees in this study, was the density of planting. Also here, negative regressions were found between the numbers of trees planted per unit of area and both trunk cross-section area and canopy volume (Figs. 3 and 4). Doubling of tree density compared with average tree spacing in the orchard reduced mean tree vigour by approximately more than one third.

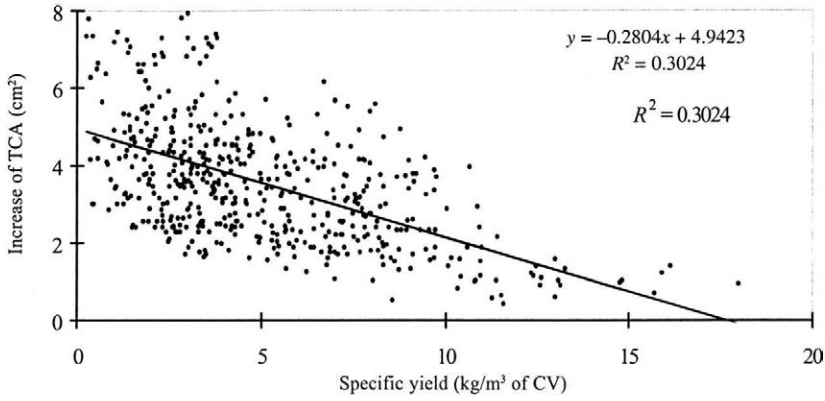


Fig. 1. Regression of trunk cross-section area on productivity of trees

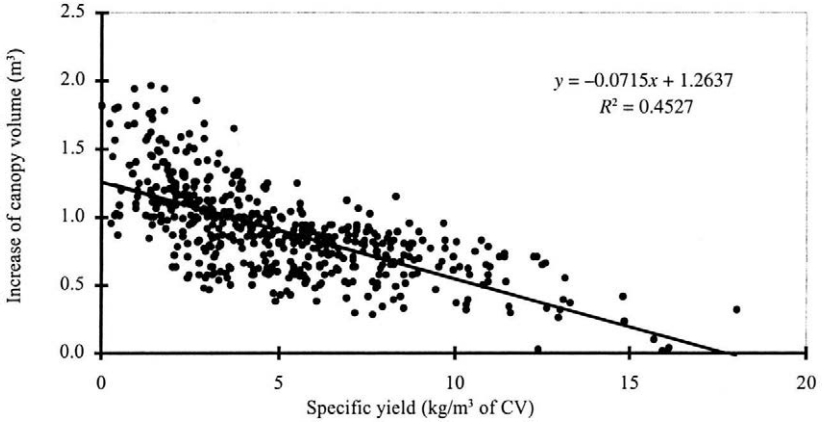


Fig. 2. Regression of canopy volume on productivity of trees

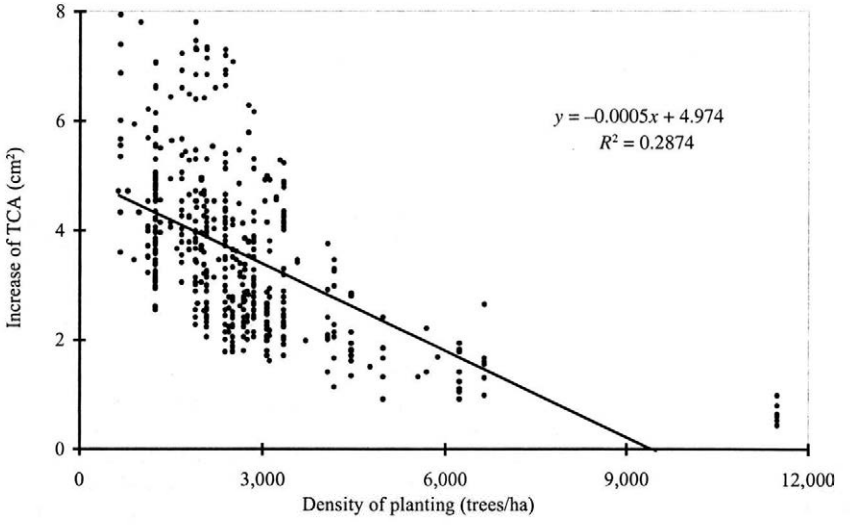


Fig. 3. Regression of trunk cross section area on density of planting

Table 4. Effect of cultivars and climatic area on specific yield (kg/m³ of canopy volume)

| Cultivar | Climatic area | | | Mean |
|---|---|---|---|---|
| | cold | medium | warm | |
| Angold | 3.51 | 4.63 | 4.03 | 4.06 |
| Bohemia | 1.41 | 1.75 | 2.01 | 1.72 |
| Braeburn | 3.40 | 4.50 | 4.27 | 4.06 |
| Delor | 3.97 | 4.00 | 4.84 | 4.27 |
| Elstar | 2.47 | 4.03 | 3.91 | 3.47 |
| Florina | 1.56 | 3.68 | 3.53 | 2.92 |
| Gala | 3.16 | 3.63 | 3.96 | 3.58 |
| Gloster | 3.58 | 4.95 | 5.59 | 4.71 |
| Golden Delicious | 3.59 | 4.42 | 4.58 | 4.20 |
| Goldstar | 3.96 | 3.98 | 4.02 | 3.99 |
| Granny Smith | 2.03 | – | 3.21 | 2.62 |
| Idared | 3.89 | 4.56 | 4.83 | 4.43 |
| Jarka | 4.57 | 4.62 | 4.36 | 4.51 |
| Jonagold | 3.57 | 3.94 | 4.62 | 4.04 |
| Jonalord | 5.71 | 6.42 | 4.91 | 5.68 |
| Julia | 2.33 | 3.20 | 3.62 | 3.05 |
| Karmína | 4.00 | 4.27 | 5.21 | 4.49 |
| Melodie | 4.06 | 4.72 | 5.43 | 4.74 |
| Melrose | 2.27 | 3.16 | 4.15 | 3.19 |
| Otava | 4.93 | 5.28 | 5.05 | 5.09 |
| Produkta | 4.76 | 6.39 | 7.30 | 6.15 |
| Rajka | 4.27 | 4.74 | 4.57 | 4.53 |
| Resista | 3.30 | 3.61 | 4.15 | 3.69 |
| Rosana | 3.21 | 4.14 | 4.49 | 3.95 |
| Rubín | 1.56 | 1.83 | 1.87 | 1.75 |
| Rubinola | 1.76 | 1.80 | 2.02 | 1.86 |
| Selena | 3.49 | 4.60 | 5.69 | 4.59 |
| Šampion | 4.84 | 4.81 | 5.13 | 4.93 |
| Topaz | 4.27 | 4.40 | 5.66 | 4.78 |
| Vanda | 4.79 | 4.70 | 4.28 | 4.59 |
| Zuzana | 2.51 | 3.18 | 2.40 | 2.70 |
| Total | 3.44 | 4.16 | 4.47 | 4.06 |
| $LSD_{0.05}$ | 0.43 | 0.31 | 0.32 | – |

## DISCUSSION

Within the chosen assortment of apples evaluated on M 9 rootstock in different growing conditions in the Czech Republic, the cultivar was the main factor, which determined the vigour of apple-trees in orchards. Cultivars with the least tree vigour (Braeburn and Delor) grew more than 50% weaker than the most vigorous ones (Bohemia and Rubín). This span of differences more or less coincides with the variation of apple tree vigour that was found in this country 20 years ago (PAPRŠTEIN et al. 1983). Nevertheless, in that study Boskoop was evaluated as the most vigorous, while the weakest vigour was found in Goldenspur.

A large part of the differences in tree vigour of given cultivars, however, was related to differences in their yields. A relation holds generally, that the more productive a tree is, the smaller is its vigour and vice versa. In the course of further study hereof suggested with particular tree cultivars the vigour of non-bearing trees should be separated from their vigour in times of full cropping.

Šampion, Golden Delicious and Idared, which are the most common cultivars in the Czech Republic, were characterized by medium tree vigour. On the other hand, cultivars Rubín and Bohemia, which have been frequently planted recently in the Czech Republic, are very vigorous.

The rank of cultivars, according to tree vigour determined in this study, mostly coincide with data in litera-

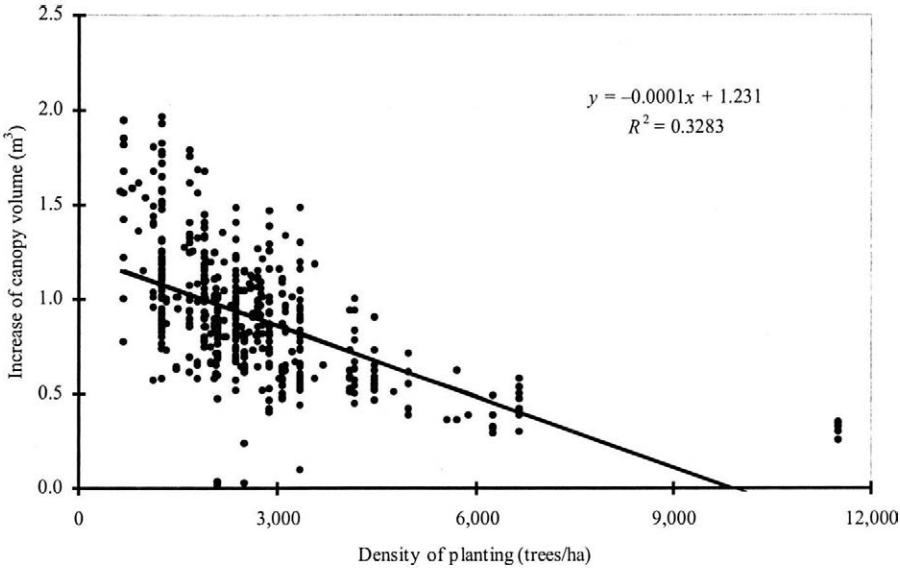


Fig. 4. Regression of canopy volume on density of planting

ture (FISCHER 1995; RIESEN et al. 1980; RUEß 2000; WAY 1979). In some cases, however, there are distinct discrepancies. For example, Melrose was assessed by GRASLUND (1989) as a medium vigorous cultivar only, and the vigour of Rubín was classified by the author as weak. These considerable differences may be due entirely to diverse climatic conditions.

Another important factor that influenced tree vigour in this study was the density of planting, hereto defined by the number of trees planted on one hectare of orchard area. Despite the fact that the number evaluated in densities over 4,000 tree/ha was relatively low, it was found unambiguously, that these densities had a very strong influence on the reduction of tree vigour. Preliminary results however indicate, thereon, that the response of single cultivars on these extreme densities is not the same.

The results concerning the influence of planting density on tree vigour, which were found in this work, generally agree with findings in literature. For example, WIDMER and KREBS (2000) concluded that doubling the density from 3,000 to 6,000 trees per hectare decreased tree vigour by 45%. MIKA and KRAWIEC (1999) stated that in the case of semi-dwarf rootstocks, an even less span of density decreased trunk cross-section area more considerably.

Climatic conditions were also a factor of great influence on the vigour of apple-trees. In warmer regions of lower elevations, trees were generally more vigorous than those growing in "cold" conditions of higher altitudes. With cultivars requiring a longer growing season such as Granny Smith, these differences were greater than in the case of early ripening cultivars. Likewise, even among cultivars with usually higher yields in warm regions (for example Jonagold), the differences were negligible.

The growth rate of trees in apple orchards affects a range of additional factors (PAPRŠTEIN et al. 1983), which however were not subjects of this study, including properties of soil, amount of rainfall, method of soil management, and other effects. The quality of planting materials also has significant impact (PONIEDZIALEK et al. 1995).

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## Vzrůstnost stromů nových odrůd jabloní pěstovaných v České republice a faktory, které ji ovlivňují

**ABSTRAKT**: Vzrůstnost jabloní na podnoži M 9 byla sledována v letech 1996–2000 v 35 výsadbách, které se nacházejí v různých klimatických podmínkách České republiky. Celkem 31 odrůd, nejčastěji vysazovaných nebo v předcházejících letech považovaných za perspektivní pro zemi, bylo seřazeno podle hodnoty syntetického růstového indexu, který byl založen na průměrném přírůstku plochy příčného průřezu kmene, objemu koruny a délce jednoletých výhonů. Odrůdy s nejmenší vzrůstností stromů (Braeburn a Delor) rostly o více než 50 % slaběji než kultivary s nejsilnějším růstem (Bohemia a Rubín). Značný podíl rozdílů ve vzrůstnosti stromů mezi odrůdami však souvisel s rozdíly v jejich výnosech. Dalším důležitým faktorem, který ovlivňoval intenzitu růstu stromů v tomto studiu, byla hustota výsadby. Na vzrůstnost jabloní měly významný vliv i klimatické podmínky. V teplých oblastech s nižší nadmořskou výškou rostly stromy většiny odrůd silněji než v chladnějších podmínkách s vyšší nadmořskou výškou. Některé odrůdy však reagovaly na odlišné klimatické podmínky slaběji než jiné.

**Klíčová slova**: jabloň; odrůdy; vzrůstnost stromů; klimatické podmínky; hustota výsadby; podnož M 9; korelace

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# The influence of tree decline on yields of new genotypes of apricots and some cultivars of the world collection (*Prunus armeniaca* L.)

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**ABSTRACT**: Tree decline and fruit-bearing were evaluated in an experimental orchard with 21 apricot genotypes on M-LE-1 rootstock (*Prunus armeniaca* L.) from the year of planting in 1993 to 2000. The Czech cultivar Velkopavlovická and three cultivars of the world collection (Hargrand, Salah and Bergeron LE-2) were used as controls. Fifty trees of each genotype were planted in a long row. Ideal yield (by the full number of trees per hectare) and real yield (inclusive of actual decline of trees) were evaluated each year. There were normal conditions for apricot fruit-bearing only in 1996 and 1999 over the period 1996–2000. Significantly lower fruit-bearing was typical of the other years. A bumper crop was obtained in 1999, when the average per-hectare yield amounted to 24.3 t/ha (17.5 t/ha in the control cultivar Velkopavlovická). Real yields per hectare, inclusive of the actual decline, were also high at that year (18.4 t/ha on average). The crop was low in the years less favorable for fruit-bearing, many times below 1.0 t/ha. In spite of this, the real average crop over 1996–2000 was 5.7 t/ha. A highly significant correlation ($r = 0.92^{++}$) was determined between ideal and real yields per hectare. The percentage of tree decline, sometimes called vitality, was not in a significant correlation with individual fruit-bearing per tree in kg. The highest-yielding genotypes were those with highest proportions of crops in total fruit-bearing in unfavorable years (Lejuna, LE-498, Legolda, LE-4725 and Leala).

**Keywords**: apricot; genotype; fruit-bearing; decline

Cultivar is a key factor of apricot production. It is not easy to achieve both the high quality of fruits and the stability of fruit-bearing (BASSI 1999). New and new cultivars are bred throughout the world, having a different level of required characteristics, different fruit-bearing and suitability for a specific production area (AUDERGON et al. 1999; BLAŽKOVÁ 1999; HOFSTEE et al. 1999; PENNONE 1999; SYRGIANIDIS, MAINOU 1999; VACHŮN et al. 1999). Fruit-bearing of cultivars per unit area is substantially influenced by a premature decline of trees. The species *Prunus armeniaca* L. developed in the continental conditions of Central and Northern China. After the species was brought to Central Europe, it was transferred to areas with large temperature fluctuations in a post-dormancy period and with different distribution of precipitation in winter and summer seasons. The negative effect of such changes in the environment is enhanced by pathogenic agents (fungi, bacteria, viroses, and phytoplasmoses in recent time – MORVAN 1977; ROZSNAY, KLEMENT 1973). The objective of research is to find genotypes resistant to different pathogens (PRUNIER et al. 1999); it could lead to a decrease in premature decline and to an increase in yield per unit area. The genotypes differ in their individual fruit-bearing. But the evaluation of 17 selected apricot hybrids over a 15-year period did not demonstrate any significant correlation between decline percentage and individual fruit-bearing (PLAZINIČ et al. 1999). Long-term observations are necessary for a complex assessment of biotic and abiotic factors.

The objective of the paper was to evaluate the influence of tree decline on yield per unit area in new cultivars and hybrids and in some cultivars of the world collection of apricots in the first eight years after planting.

## MATERIAL AND METHODS

Research was conducted in an orchard with 21 apricot cultivars and hybrids on the rootstock M-LE-1 (*Prunus armeniaca* L.). The evaluation was carried out from 1993, when the experimental orchard was established, to 2000. Velkopavlovická LE-12/2 was a control cultivar. For the purposes of comparison, another three cultivars from the world collection of apricots were planted – Bergeron LE-2, Salah (syn. Erevani) and Hargrand. The other genotypes (cultivars and hybrids) included in the experiment are of Czech origin. They were bred at the Lednice-based Faculty of Horticulture of Mendel University of Agriculture and Forestry Brno.

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Table 1. The crop weight of apricot genotypes in t/ha by the full number of trees (500 individuals per hectare, 5 × 4 m spacing) in the first five years from onset of commercial fruit-bearing

| Genotype | Crop (t/ha) in | | | | | Crop over 5 years | |
|---|---|---|---|---|---|---|---|
| | 1996 | 1997 | 1998 | 1999 | 2000 | total | average |
| Lebela (LE-1309) | 3.2 | 0.1 | 2.0 | 16.5 | 0.2 | 21.9 | 4.4 |
| LE-3662 | 2.4 | 0.2 | 0.3 | 19.5 | 0.1 | 22.3 | 4.5 |
| LE-3709 | 5.2 | 0.1 | 0.1 | 22.5 | 0.1 | 27.9 | 5.6 |
| **Velkopavlovická LE-12/2** | **0.3** | **0.1** | **0.3** | **17.5** | **10.0** | **28.1** | **5.6** |
| **Šalach** | **1.7** | **0.2** | **0.3** | **24.5** | **3.5** | **30.1** | **6.0** |
| Lemira (LE-1446) | 3.0 | 3.7 | 0.3 | 21.0 | 3.0 | 30.9 | 6.2 |
| **Hargrand** | **3.9** | **1.7** | **0.3** | **25.5** | **0.1** | **31.4** | **6.3** |
| LE-3255 | 4.4 | 0.5 | 4.5 | 24.0 | 0.3 | 33.7 | 6.7 |
| **Bergeron LE-2** | **1.9** | **1.5** | **2.5** | **24.0** | **4.0** | **33.9** | **6.8** |
| Leskora (LE-836) | 5.8 | 0.6 | 3.5 | 23.5 | 0.5 | 33.9 | 6.8 |
| Lednická (M 90 A) | 1.6 | 0.7 | 1.3 | 25.0 | 6.0 | 34.6 | 6.9 |
| Lebona (LE-984) | 2.9 | 0.7 | 0.3 | 30.0 | 1.0 | 34.9 | 7.0 |
| LE-3204 | 6.4 | 0.3 | 1.0 | 27.5 | 0.5 | 35.7 | 7.1 |
| Lerosa (LE-1328) | 4.6 | 1.4 | 0.2 | 27.5 | 3.5 | 37.1 | 7.4 |
| Lefreda (LE-833) | 6.4 | 0.7 | 3.0 | 28.0 | 0.3 | 38.4 | 7.7 |
| Ledana (LE-1041) | 6.3 | 0.9 | 0.5 | 32.5 | 0.1 | 40.2 | 8.0 |
| Leala (LE-352) | 1.3 | 2.1 | 6.0 | 18.5 | 12.5 | 40.4 | 8.1 |
| Legolda (LE-980) | 2.3 | 1.3 | 8.0 | 30.0 | 0.5 | 42.1 | 8.4 |
| LE-4725 | 6.4 | 0.3 | 12.5 | 22.5 | 3.0 | 44.7 | 8.9 |
| LE-498 | 0.3 | 2.8 | 7.0 | 25.5 | 10.0 | 45.6 | 9.1 |
| Lejuna (LE-805) | 5.3 | 0.9 | 16.0 | 24.0 | 7.5 | 53.6 | 10.7 |
| Average | 3.6 | 1.0 | 3.3 | 24.3 | 3.2 | 35.3 | 7.1 |

Data in bold – control varieties

The growth habit was a bush tree (stem height 0.7 m) with hollow crown. Tree spacing 5 × 4 m. Fifty trees of each genotype were planted in long rows. The number of trees was lower by 1, 4 and 9 trees in three cases only. No underplantings were carried out in subsequent years. Identical cuts and cultural practices were employed in the whole orchard. Drip irrigation and fertilizing on the basis of soil analyses were used.

Tree declines were recorded each year from the orchard establishment. Decline is taken to mean total decline, i.e. decline of the whole tree, not a partial decline (e.g. a third or a half of the crown). Yield was evaluated by estimating fruit weight.

In the present paper, ideal yield per hectare is taken to mean the yield provided by the full number of trees per 1 hectare. Real yield per hectare is taken to mean the yield inclusive of the actual decline of trees per unit area of one hectare.

Unistat program was used for statistical data processing in form of correlation coefficients and multiple comparison after preceding assessment of variance homogeneity.

## RESULTS AND DISCUSSION

In the period 1996–2000, normal crops of apricots were produced in 1996 and 1999. Lower yields were recorded in three years (1997, 1998 and 2000). They were caused by adverse weather conditions at the beginning of growing seasons in 1997 and 1998. The yield in 2000 was lower due to tree exhaustion after a bumper production in 1999, when the average calculated ideal yield per hectare (by the full number of trees per hectare) was 24.3 t/ha. The highest crops were produced by the genotypes Ledana (32.5 t/ha), and Legolda and Lebona (30.0 t/ha). High yields were also achieved by control cultivars Velkopavlovická (17.5 t/ha), Bergeron (24 t/ha) and Salah (24.5 t/ha) that year. The crop of a number of genotypes was below 1.0 t/ha in years with unfavorable conditions: e.g. in hybrids LE-3662, LE-3709, cultivars Hargrand, Lebela or in control cv. Velkopavlovická. This is the reason why the average ideal annual yield over five years was substantially lower due to three years with low crop. The highest average per-hectare yield in the years 1996–2000 was produced by Lejuna (10.7 t/ha), LE-498 (9.1 t/ha) and LE-4725 (8.9 t/ha) (Table 1).

Real yields per hectare, inclusive of the actual tree decline, were lower in the particular years. The average real crop was 18.4 t/ha in 1999. The highest crops were achieved by Lebona (25.8 t/ha) and Ledana (24.7 t/ha). The average real crop over the years 1996–2000 was 5.7 t/ha. Although the period was less favorable in view of apricot fruit-bearing, the average crop in question was

Table 2. The crop of apricots in t/ha from the onset of commercial fruit-bearing in 1996–2000 inclusive of tree decline in the years of observation

| Genotype | Crop (t/ha) in | | | | | Crop over 5 years | |
|---|---|---|---|---|---|---|---|
| | 1996 | 1997 | 1998 | 1999 | 2000 | total | average |
| LE-3709 | 4.99 | 0.08 | 0.07 | 12.15 | 0.05 | 17.34 | 3.5 |
| LE-3662 | 2.35 | 0.17 | 0.24 | 15.60 | 0.06 | 18.42 | 3.7 |
| Lebela (LE-1309) | 3.20 | 0.10 | 1.96 | 14.52 | 0.13 | 19.91 | 4.0 |
| Lemira (LE-1446) | 3.00 | 2.74 | 0.20 | 12.60 | 1.80 | 20.34 | 4.1 |
| **Hargrand** | **3.74** | **1.60** | **0.26** | **15.81** | **0.06** | **21.47** | **4.3** |
| LE-3255 | 4.22 | 0.46 | 4.14 | 14.88 | 0.19 | 23.89 | 4.8 |
| **Velkopavlovická LE-12/2** | **0.30** | **0.10** | **0.29** | **15.40** | **8.80** | **24.89** | **5.0** |
| **Šalach** | **1.70** | **0.20** | **0.29** | **20.02** | **2.79** | **25.00** | **5.0** |
| LE-3204 | 6.40 | 0.28 | 0.88 | 19.25 | 0.32 | 27.13 | 5.4 |
| Lerosa (LE-1328) | 4.60 | 1.29 | 0.16 | 19.25 | 2.38 | 27.68 | 5.5 |
| **Bergeron LE-2** | **1.90** | **1.50** | **2.45** | **20.64** | **3.36** | **29.85** | **6.0** |
| Lefreda (LE-833) | 6.21 | 0.62 | 2.46 | 20.72 | 0.22 | 30.23 | 6.0 |
| Lebona (LE-984) | 2.78 | 0.66 | 0.28 | 25.80 | 0.86 | 30.38 | 6.1 |
| Leskora (LE-836) | 5.66 | 0.54 | 3.16 | 21.20 | 0.43 | 30.99 | 6.2 |
| Lednická (M 90 A) | 1.60 | 0.69 | 1.25 | 22.50 | 5.28 | 31.32 | 6.3 |
| Ledana (LE-1041) | 6.30 | 0.86 | 0.46 | 24.70 | 0.06 | 32.38 | 6.5 |
| Leala (LE-352) | 1.30 | 2.10 | 5.88 | 14.06 | 9.50 | 32.84 | 6.6 |
| LE-4725 | 6.12 | 0.27 | 10.60 | 14.18 | 1.89 | 33.06 | 6.6 |
| Legolda (LE-980) | 2.21 | 1.14 | 7.04 | 24.00 | 0.38 | 34.77 | 7.0 |
| LE-498 | 0.30 | 2.74 | 6.58 | 21.42 | 8.40 | 39.44 | 7.9 |
| Lejuna (LE-805) | 5.09 | 0.86 | 15.04 | 17.76 | 5.55 | 44.30 | 8.9 |
| Average | 3.52 | 0.90 | 3.03 | 18.40 | 2.50 | 28.36 | 5.67 |

Note: The only cultivar Hargrand bore fruit in 1995 (2 t/ha) and this crop was included in 1996 crop
Data in bold – control varieties

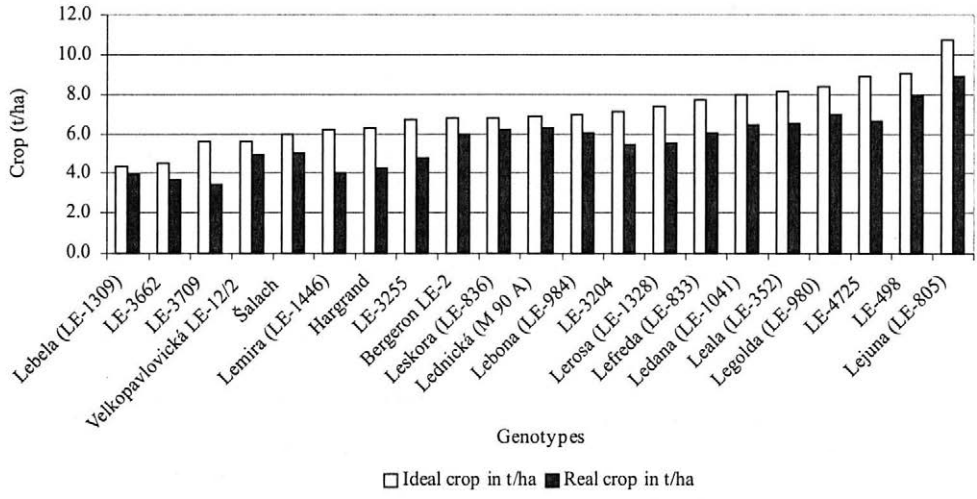


Fig. 1. Average ideal crops per hectare exclusive of tree decline and real yields per hectare inclusive of tree decline in 1996–2000. Correlations between the rank of genotypes ($r = 0.92^{**}$)

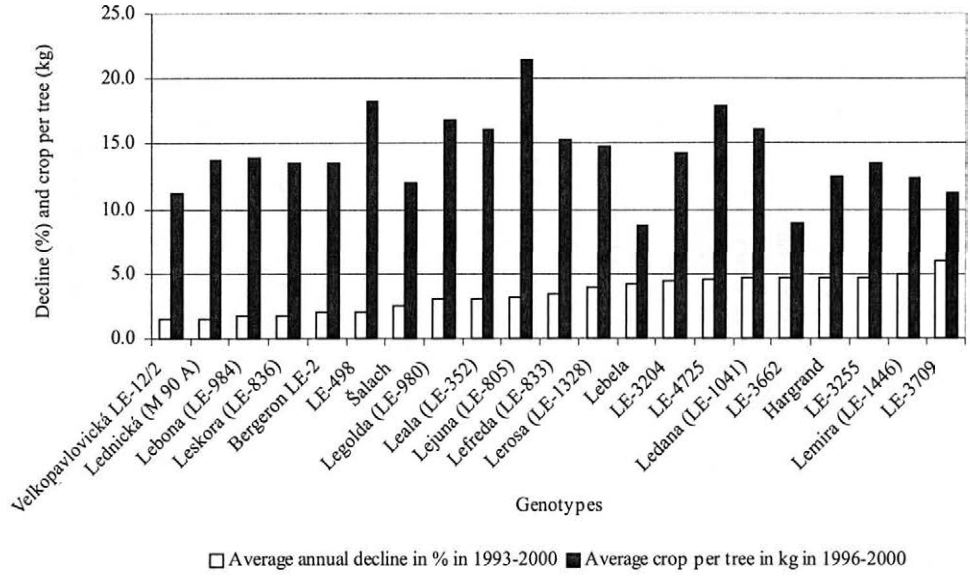


Fig. 2. Genotype vitality expressed as average annual percentage of decline and average crop per tree in kg (correlation coefficient $r = 0.18$)

Table 3. The sum of apricot crops in t/ha and in per cent inclusive of tree declines in normal-crop years and in low-fruit-bearing years

| Genotype | Crop sums (t/ha) in | | | Crop sums (%) in | | |
|---|---|---|---|---|---|---|
| | 1996 to 2000 in total | 1996 and 1999 with normal fruit-bearing | 1997, 1998 and 1999 with lower fruit/bearing | 1996 to 2000 in total | 1996 and 1999 with normal fruit-bearing | 1997, 1998 and 1999 with lower fruit/bearing |
| LE-3709 | 17.34 | 17.14 | 0.20 | 100.00 | 98.85 | 1.15 |
| LE-3662 | 18.42 | 17.95 | 0.47 | 100.00 | 97.45 | 2.55 |
| Lebela (LE-1309) | 19.91 | 17.72 | 2.19 | 100.00 | 89.00 | 11.00 |
| Lemira (LE-1446) | 20.34 | 15.60 | 4.74 | 100.00 | 76.70 | 23.30 |
| **Hargrand** | **21.47** | **19.55** | **1.92** | **100.00** | **91.05** | **8.94** |
| LE-3255 | 23.89 | 19.10 | 4.79 | 100.00 | 79.95 | 20.05 |
| **Velkopavlovická LE-12/2** | **24.89** | **15.70** | **9.19** | **100.00** | **63.08** | **36.92** |
| **Šalach** | **25.00** | **21.72** | **3.28** | **100.00** | **86.88** | **13.12** |
| LE-3204 | 27.13 | 25.65 | 1.48 | 100.00 | 94.54 | 5.46 |
| Lerosa (LE-1328) | 27.68 | 23.85 | 3.83 | 100.00 | 86.16 | 13.84 |
| **Bergeron LE-2** | **29.85** | **22.54** | **7.31** | **100.00** | **75.51** | **24.49** |
| Lefreda (LE-833) | 30.23 | 26.93 | 3.30 | 100.00 | 89.08 | 10.92 |
| Lebona (LE-984) | 30.38 | 28.58 | 1.80 | 100.00 | 94.08 | 5.92 |
| Leskora (LE-836) | 30.99 | 26.86 | 4.13 | 100.00 | 86.67 | 13.33 |
| Lednická (M 90 A) | 31.32 | 24.10 | 7.22 | 100.00 | 76.95 | 23.05 |
| Ledana (LE-1041) | 32.38 | 31.00 | 1.38 | 100.00 | 95.74 | 4.26 |
| Leala (LE-352) | 32.84 | 15.36 | 17.48 | 100.00 | 46.77 | 53.23 |
| LE-4725 | 33.06 | 20.30 | 12.76 | 100.00 | 61.40 | 38.60 |
| Legolda (LE-980) | 34.77 | 26.21 | 8.56 | 100.00 | 75.38 | 24.62 |
| LE-498 | 39.44 | 21.72 | 17.72 | 100.00 | 55.07 | 44.93 |
| Lejuna (LE-805) | 44.30 | 22.85 | 21.45 | 100.00 | 51.58 | 48.42 |

Data in bold – control varieties

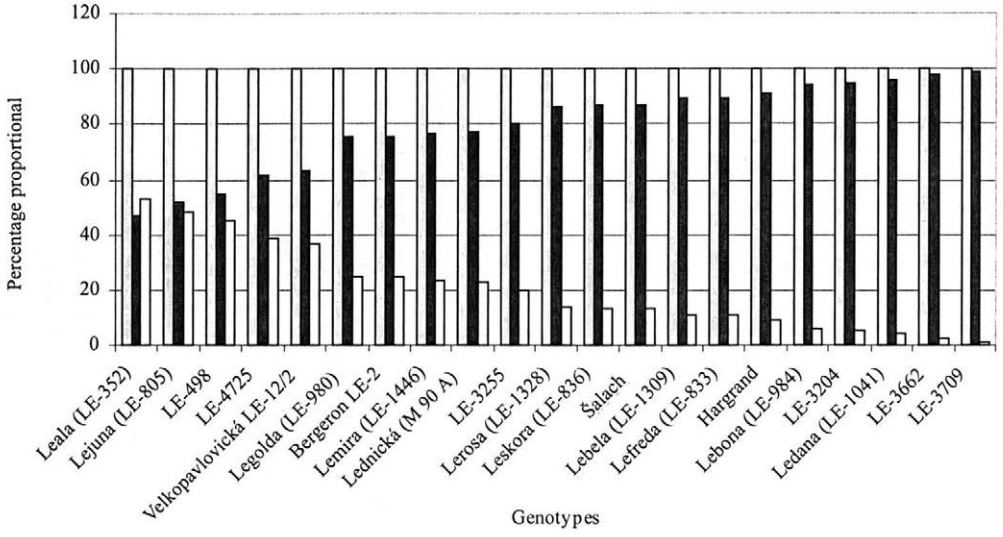


Fig. 3. Percentage proportions of real crop weights per 1-ha area in normal fruit-bearing years and in lower-fruit bearing years over 1996–2000 (real crops = crops inclusive of tree decline per 1-ha area)

twice higher than the average production in the Czech Republic over 10 years, and by 30% higher than the long-term average. Some genotypes outyielded the long-term average 2.5 times (Lejuna 10.7 t/ha) (Table 2).

The correlation between ideal (by the full number of trees per hectare) and real (inclusive of the tree decline) yields per hectare was highly significant as documented by the correlation coefficient $r = 0.92^{++}$ (Fig. 1).

The percentage of decline, as a genotype trait, is sometimes called vitality, i.e. the capacity of the genotype to survive under the given conditions. Such vitality in the evaluated collection of apricots is not in a significant

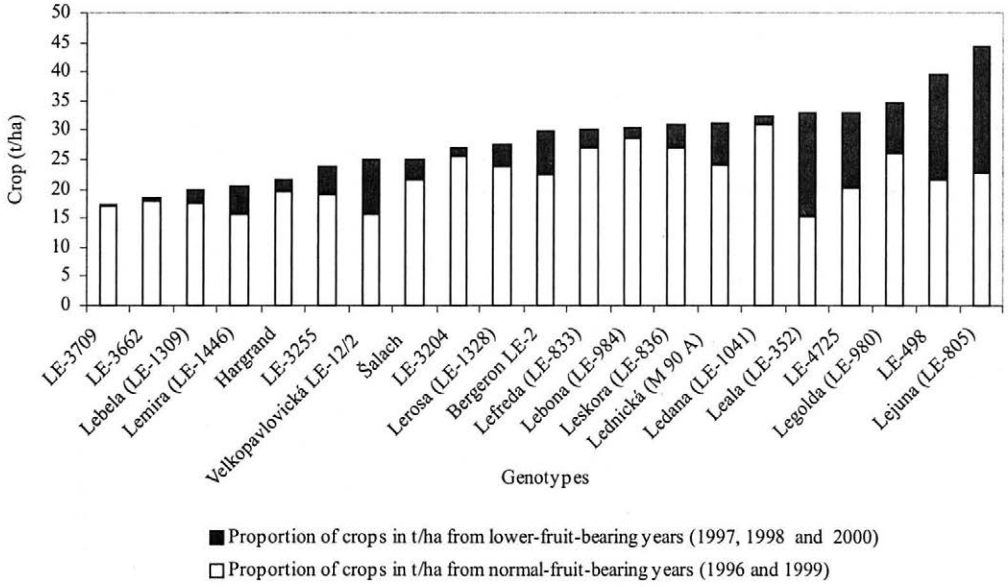


Fig. 4. The sum of real crops of apricot genotypes in t/ha over five years and the proportion of crops from normal-fruit bearing years (1996 and 1999) and from lower-fruit-bearing years (1997, 1998 and 2000)

Table 4. Evaluation of the significance of differences in the crops of apricot genotypes in lower fruit-bearing years (1997, 1998 and 2000)

Multiple comparisons (Tukey's-HSD test)
Method: 95% Tukey's-HSD interval
*designates significantly different pairs. Homogeneous subgroups are in vertical columns

| Group | Cases | Ln (variance) | LE-3709 | LE-3662 | LE-3204 | Ledana (LE-1041) | Hargrand | Bergeron LE-2 | Lebela (LE-1309) | Lerosa (LE-1328) | Lefreda (LE-833) | Lemira (LE-1446) | Šalach | Leskora (LE-836) | LE-3255 | Lednická (M 90 A) | LE-498 | Legolda (LE-980) | Leala (LE-352) | Velkopavlovická LE-12/2 | LE-4725 | Lejuna (LE-805) | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| LE-3709 | 2 | –7.67 | | | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | * | I |
| LE-3662 | 2 | –4.11 | | | | | | | | | * | * | * | * | * | * | * | * | * | * | * | * | II |
| LE-3204 | 2 | –1.49 | * | | | | | | | | | | | | | | | | | * | * | * | II |
| Ledana (LE-1041) | 2 | –1.14 | * | | | | | | | | | | | | | | | | | * | * | * | II |
| Hargrand | 2 | 0.34 | * | | | | | | | | | | | | | | | | | | | | III |
| Bergeron LE-2 | 2 | 0.55 | * | | | | | | | | | | | | | | | | | | | | III |
| Lebela (LE-1309) | 2 | 0.82 | * | | | | | | | | | | | | | | | | | | | | III |
| Lerosa (LE-1328) | 2 | 0.90 | * | | | | | | | | | | | | | | | | | | | | III |
| Lefreda (LE-833) | 2 | 1.05 | * | * | | | | | | | | | | | | | | | | | | | II |
| Lemira (LE-1446) | 2 | 1.19 | * | * | | | | | | | | | | | | | | | | | | | II |
| Šalach | 2 | 1.46 | * | * | | | | | | | | | | | | | | | | | | | II |
| Leskora (LE-836) | 2 | 1.56 | * | * | | | | | | | | | | | | | | | | | | | II |
| LE-3255 | 2 | 2.28 | * | * | | | | | | | | | | | | | | | | | | | II |
| Lednická (M 90 A) | 2 | 2.53 | * | * | | | | | | | | | | | | | | | | | | | II |
| LE-498 | 2 | 2.82 | * | * | | | | | | | | | | | | | | | | | | | II |
| Legolda (LE-980) | 2 | 3.28 | * | * | | | | | | | | | | | | | | | | | | | II |
| Leala (LE-352) | 2 | 3.31 | * | * | | | | | | | | | | | | | | | | | | | II |
| Velkopavlovická LE-12/2 | 2 | 3.90 | * | * | * | * | | | | | | | | | | | | | | | | | I |
| LE-4725 | 2 | 4.12 | * | * | * | * | | | | | | | | | | | | | | | | | I |
| Lejuna (LE-805) | 2 | 4.65 | * | * | * | * | | | | | | | | | | | | | | | | | I |

Homogeneous subgroups:
Group 1: LE-3709, LE-3662
Group 2: LE-3662, LE-3204, Ledana, Hargrand, Bergeron, Lebela, Lerosa
Group 3: LE-3204, Ledana, Hargrand, Bergeron, Lebela, Lerosa, Lefreda, Lemira, Šalach, Leskora, LE-3255, Lednická, LE-498, Legolda, Leala
Group 4: Hargrand, Bergeron, Lebela, Lerosa, Lefreda, Lemira, Šalach, Leskora, LE-3255, Lednická, LE-498, Legolda, Leala, Velkopavlovická, LE-4725, Lejuna

correlation with the level of individual fruit-bearing of the genotypes. It is in accordance with the conclusions drawn by PLAZINIČ et al. (1999), that significant differences in the percentage of decline do not imply significant differences in individual fruit-bearing (Fig. 2).

Crop variations are not good for any fruit-bearing plant nor for any producer. Large fluctuations of fruit-bearing cause an undesirable stress to the plant. And producers incur profit losses. In the years of overproduction, the loss is a result of undesirable accumulation of lower-quality products and difficult marketing. In the years with low fruit-bearing, low returns are caused by low crops. It is necessary that fair crops be achieved also in the years less favorable for apricot fruit-bearing. New cultivars can play an important role in this aspect. The selection of suitable genotypes can be based on the ratio of fruit-bearing in generally unfavorable years to fruit-bearing in favorable years. The ratio of years with normal crop to those with lower crop was 2:3 in the evaluated five-year period. It is to note that the sum of crops for these two groups of years is practically identical in some genotypes. E.g. the per cent ratio for Lejuna was 51.58:48.42, for LE-498 55.07:44.93 and for Leala 46.77:53.23. This relation is described by the ratio 98.85%:1.15% in some less suitable genotypes (e.g. hybrid LE-3709) (Table 3 and Fig. 3).

Lejuna and LE-4725 were significantly higher-yielding than Velkopavlovická cv. in the tree years with low fruit-bearing. Both genotypes significantly outyielded the

fruit-bearing of some other genotypes under less favorable conditions, among them e.g. the well-known cultivars Bergeron, Hargrand or Salah (Table 4).

The most productive genotypes in the evaluated set are those with highest share of crops from unfavorable years in their total fruit-bearing. They include e.g. Lejuna, LE-498, Legolda, LE-4725 and Leala. Velkopavlovická and Bergeron were medium-yielding cultivars with a medium share of fruit-bearing in less favorable years.

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## Vliv předčasného úhynu stromů na výnosy nových genotypů meruněk a některých odrůd světového sortimentu (*Prunus armeniaca* L.)


**ABSTRAKT**: Ve výsadbě 21 genotypů meruněk na podnoži M-LE-1 (*Prunus armeniaca* L.) byl od založení v r. 1993 do roku 2000 hodnocen úhyn stromů a plodnost. Kontrolní odrůdou byla Velkopavlovická LE-12/2 a tři odrůdy ze světového sortimentu (Hargrand, Šalach a Bergeron LE-2). Každý genotyp byl vysazen v dlouhém dílci s 50 stromy. Byl vyhodnocen ideální výnos (při plném počtu rostlin na hektar) a reálný výnos (respektující skutečný úhyn stromů). V období 1996–2000 byly zcela normální podmínky pro plodnost meruněk v r. 1996 a 1999. V ostatních letech byla plodnost významně snížená. Rekordní sklizeň byla v r. 1999. V tomto roce byl průměrný hektarový výnos v celé výsadbě při plném počtu stromů (ideální výnos) 24,3 t/ha (kontrola 17,5 t/ha). Reálné hektarové výnosy, respektující skutečný úhyn, v tomto roce byly rovněž vysoké (průměr 18,4 t/ha). V letech pro plodnost méně příznivých byla sklizeň nízká a ve více případech nedosahovala ani 1,0 t/ha. Přesto reálná průměrná sklizeň za období 1996–2000 byla 5,7 t/ha, což je dvakrát více, než je průměrná sklizeň za posledních deset let v ČR a o 30 % vyšší, než je dlouhodobý průměr. Některé genotypy překonávaly tento dlouhodobý průměr 2,5krát (Lejuna 10,7 t/ha). Mezi hektarovými výnosy ideálními a reálnými byl zjištěn průkazný korelační vztah ($r$ ,= 0,92$^{++}$). Procento úhynu stromů, označované někdy jako vitalita, nekoreluje průkazně s individuální plodností v kg na strom. Nejúrodnější genotypy byly ty, na jejichž celkové plodnosti se nejvíce podílely sklizně v nepříznivých letech. Byly to Lejuna, LE-498, Legolda, LE-4725 a Leala.


**Klíčová slova**: meruňka; genotyp; plodnost; úhyn


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# Immobilized plant cells in the biotransformation of some precursors of poppy alkaloids and glycosides

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**ABSTRACT**: *Papaver somniferum* L. (opium poppy) cells were immobilized after permeabilization in Tween 80 with glutaraldehyde without any soluble carrier. Cells immobilized by cross-linking performed the biotransformation of L-DOPA and L-tyrosine for three months, without significant loss of activity of L-DOPA decarboxylase and L-tyrosine decarboxylase, resp. The immobilized cells, having high α- and ß-galactosidase activity and long-term storage stability showed appropriate physico-mechanical properties. In this way immobilized cells can be exploited for biotransformation reactions of precursors of pharmaceutically important substrates – i.e. opium alkaloids and some glycosides, resp.

**Keywords**: cell immobilization; L-DOPA and L-tyrosine decarboxylase; α- and ß-decarboxylase; α- and ß-galactosidase; *Papaver somniferum* L.

Biotransformation and production of high-value fine and special chemicals have been known since recent time. The knowledge of totipotency and mastering of plant-tissue cultivation techniques was applied at first in agriculture, for instance in plant propagation. It was found later that plant cells can be used for biosynthesis and biotransformation of various substances of natural and synthetic origin.

Immobilization represents an interesting alternative to the suspension cultivation of plant cells. It can be utilized for both the secondary metabolite production and biotransformation as well as for physiological studies. The secondary metabolites may be produced by immobilized cells de novo (e.g. antraquinones by *Morinda citrifolia* or the blue pigment by *Lavandula vera*) (Brodelius et al. 1979), from the precursors (e.g. the indole alkaloid ajmalicin from tryptamine and secologanine by *Catharanthus roseus*) or most often by biotransformation (e.g. 3-methyldigoxin from 3-methyldigitoxin by *Digitalis lanata* (Alfermann et al. 1980), 5-ß-hydroxygitoxigenin from gitoxigenin by *Daucus carota* (Jones, Veliky 1981), L-DOPA from L-tyrosine by *Mucuna pruriens* (Prass et al. 1989) or codeine from codeinone by *Papaver somniferum* (Furuya et al. 1984).

The entrapment of cells in a polymer matrix is the most widely used technique for cell immobilization. Microbial cells can be immobilized by glutaraldehyde with or without a soluble carrier. Plant cells without any soluble carrier were successfully immobilized recently (Stano et al. 1995). Tyramine and dopamine have an important role in the initial steps of isoquinoline alkaloids biosynthesis (Roberts et al. 1987).

The activity of L-DOPA decarboxylase (EC 4.1.1.26) has been demonstrated in poppy latex (Roberts, Antoun 1978; Roberts et al. 1983). Decarboxylation of aromatic amino acids L-tyrosine, L-phenylalanine and L-DOPA was also demonstrated in seedlings of poppy plants in experiments *in vitro* (Jindra et al. 1966).

α-galactosidase (α-D-galactoside galactohydrolase, EC 3.2.1.22) and ß-galactosidase (ß-D-galactoside galactohydrolase, EC 3.2.1.23) catalyse the hydrolysis of terminal α- and ß-galactosidic linkages of glycosides, resp., microorganisms are preferred sources of α- and ß-galactosidases for the production of industrial biocatalysts (Kaneko et al. 1990). Microbial biocatalysts preferentially transform L-tyrosine to L-DOPA (Prass et al. 1989). Although all above-mentioned biocatalysts are generally present also in plants, this source of enzymes has not been used previously.

In this paper, the decarboxylation of L-tyrosine to tyramine and L-DOPA to dopamine, the enzymic hydrolysis of terminal α- and ß-galactosidic linkages of glycoside by cells of *Papaver somniferum* L. immobilized by glutaraldehyde cross-linking are described.

## MATERIAL AND METHODS

### Tissue cultures

Long-term callus culture was derived from seedlings of *Papaver somniferum* L. cv. *Amarín* (Dr. K. ERDELSKÝ, Department of Plant Physiology, Comenius University, Bratislava) and continuously subcultured on Z agar medium every three weeks (ČIERNA et al. 1991). About 2–3 g of callus tissue were transferred to Z liquid medium with 0.05 ppm α-naphthaleneacetic acid and grown on a rotatory shaker (120 rpm) in 500 ml flasks, containing 100 ml of medium at 26°C in the dark. The suspension cultures were subcultured every two weeks.

### Permeabilization of the cells

Suspension culture cells (15 g wet weight) were filtered through a nylon cloth and suspended in 50 ml of 6% Tween 80 in 0.15 mol/l NaCl solution. Permeabilization proceeded for 3 h under moderate stirring at 20°C. The cells were filtered off and washed with 3,000 ml of distilled water and 1,000 ml of 0.15 mol/l NaCl solution.

### Immobilization

The permeabilized cells were immediately suspended in 50 ml of 0.15 mol/l NaCl solution, and 5 ml of 25% glutaraldehyde solution under mild stirring at laboratory temperature were added slowly. Immobilization proceeded for 2 h under moderate stirring at 20°C. The cells were filtered off and washed with 3,000 ml of distilled water and 2,000 ml of 0.15 mol/l NaCl.

### Determination of fresh and dry weight

Fresh and dry weight of suspended cells and immobilized cells was determined gravimetrically. For determination of dry weight, samples were dried to the constant weight at 100°C.

### Enzyme assay

The activities of L-tyrosine decarboxylase (TDC) and L-DOPA decarboxylase (DOPADC) were determined using L-[U-$^{14}$C] tyrosine (ÚVVVR, Czechoslovakia) and L-[1-$^{14}$C] DOPA (Amersham, England), resp.

The reaction mixture contained $2.10^{-2}$ mol/l Na phosphate buffer pH 7.2, $5.10^{-4}$ mol/l L-[1-$^{14}$C] DOPA (1.85 kBq), $5.10^{-5}$ mol/l pyridoxal-5-phosphate (PLP) (Serva, Germany) and suitable amount of immobilized cells (0.1 g/ml), or $2.10^{-2}$ mol/l Na phosphate buffer pH 8.4, $2.5.10^{-5}$ mol/l L-[U-$^{14}$C] tyrosine (4 kBq), $5.10^{-5}$ mol/l PLP and immobilized cells (0.1 g/ml) in the shaker flask. Controls contained boiled immobilized cells.

Both mixtures were kept for 3–60 min at 30°C and 80 rpm in a rotatory shaker and the reaction was stopped by adding of $NH_2OH.HCl$ (final concentration $1.10^{-3}$ mol/l) (Sigma, USA). The incubation mixtures were analysed by PC on Whatman No 1 in iso-PrOH:$NH_4OH$:water (7:1:2). Aliquots of incubation mixtures and standards of L-tyrosine, L-DOPA, tyramine (Sigma, USA) and dopamine (Koch Light, England) were identified by detection with ninhydrin reagent. Visualized spots of substrates and products were cut off, and the radioactivity was measured in a scintillation spectrometer Packard Tri-Carb (STANO et al. 1995).

The activity of α- and ß-galactosidase (α-GAL, ß-GAL) activity was determined by the modified method of SIMONS et al. (1989) using p-nitro-phenyl-α-D-galactopyranoside (α-PNG) (Serva, Germany) and p-nitro-phenyl-ß-D-galactopyranoside (ß-PNG) (Serva, Germany) as substrates. The reaction mixture contained 0.1 g of wet cells and 0.5 mg α-PNG or ß-PNG resp. in 2 ml of Mc Ilvaine buffer pH 5.6 and 4.3 resp. The mixture was incubated at 30°C for 20 min and the reaction was stopped with 2 ml of 1 mol/l $Na_2CO_3$. The control contained boiled cells. The amount of p-nitrophenol released was determined spectrophotometrically at 420 nm.

The cells were suspended from the reaction mixture, dried and enzyme activities were calculated to 1 g of dry weight.

The enzyme activity is expressed in katals. Protein contents were determined by the method of BRADFORD (1976), using bovine serum albumin (Serva, Germany) as the standard protein.

## RESULTS AND DISCUSSION

The multifunctional enzyme system of viable cells might be useful for synthesis of some plant secondary metabolites or their precursors.

Immobilization techniques have had a great impact on technology nowadays. In this work an immobilization technique without any soluble carrier was used. The cells were immobilized by cross-linking. In the cells immobilized in this way some enzyme activities had still high values even after 3 months (STANO et al. 1995, 1998).

The secondary compounds in the immobilized cells accumulate in the vacuoles and after permeabilization are liberated into the medium (FELIX et al. 1981; BRODELIUS, NILSON 1983). The permeabilization of the poppy cells by Tween 80 led to a loss of proteins while enzyme activities showed a moderate decrease (Tables 1, 2).

The microscopic investigation of the immobilized cells compared with cell suspensions showed evident morphological changes. The moderate thinning of cell walls after permeabilization was observed. Important is also the appearance of cytoplasm plasmolysis and only a moderate aggregation of cells occurring by the immobilization.

The catalytic properties of partially purified enzyme preparation from suspension cultured cells were investigated and enzyme characteristics were obtained: pH optima 8.4 for TDC, 7.2 for DOPADC, 5.4 for α-GAL and 4.8 for ß-GAL, the apparent Km value for L-tyrosine is $0.25.10^{-3}$ mol/l, $0.21.10^{-3}$ mol/l for L-DOPA. Pyridoxal-5-phosphate dependence and inhibitory effect of hydroxylamine and $HgCl_2$ were observed (WEISSOVÁ 1993). The apparent Km value for α-PNG is $3.6.10^{-4}$ mol/l and $5.6.10^{-4}$ mol/l for ß-PNG.

Table 1. The L-tyrosine decarboxylase and L-DOPA decarboxylase activities in cell suspensions and in immobilized cells

| Cells | Protein (mg/g dry weight) | Activity (n-kat/g dry weight) | | Specific activity (p-kat/mg protein) | |
|---|---|---|---|---|---|
| | | DOPADC | TDC | DOPADC | TDC |
| Suspension | 12.1 ± 0.19 | 4.84 ± 0.20 | 0.32 ± 0.21 | 400 | 26.4 |
| Permeabilized | 8.6 ± 0.21 | 4.06 ± 0.23 | 0.26 ± 0.22 | 472 | 30.2 |
| Immobilized | 8.8 ± 0.20 | 4.12 ± 0.21 | 0.27 ± 0.23 | 468 | 30.6 |

Table 2. The α- and β-galactosidase activities in cell suspensions and in immobilized cells

| Cells | Protein (mg/g dry weight) | Activity (n-kat/g dry weight) | | Specific activity (p-kat/mg protein) | |
|---|---|---|---|---|---|
| | | α-GAL | β-GAL | α-GAL | β-GAL |
| Suspension | 12.8 ± 0.20 | 4.53 ± 0.21 | 2.82 ± 0.21 | 0.35 | 0.22 |
| Permeabilized | 8.3 ± 0.19 | 7.70 ± 0.23 | 2.15 ± 0.22 | 0.44 | 0.26 |
| Immobilized | 8.2 ± 0.22 | 3.76 ± 0.21 | 2.18 ± 0.22 | 0.46 | 0.27 |

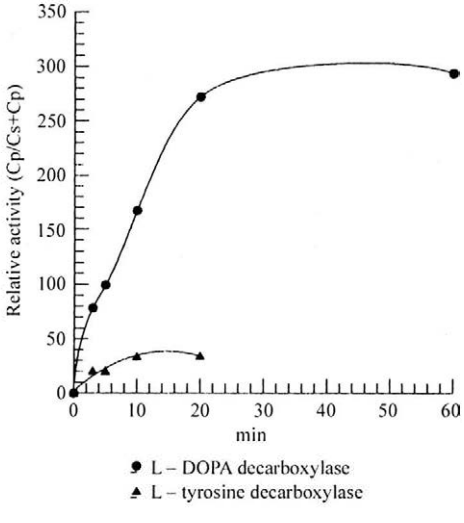


Fig. 1. Time course of L-DOPA decarboxylase and L-tyrosine decarboxylase activity in immobilized cells of *Papaver somniferum* L.

The inhibitory effect of p-chloromercurybenzoic acid can be eliminated with cysteine, dithiothreitol and 2-mercaptoethanol. These results indicate that -SH groups are essential for the enzyme activity. Partially purified enzyme preparations of α- and ß-galactosidase from gherkin and poppy seedlings were inhibited by galactose and glucose in a moderate way. A similar inhibitory effect was observed in immobilized cells, too (STANO et al. 1998).

L-DOPA decarboxylation by immobilized cells is linear for 30 min reaching 83% of conversion, then it practically stops. Decarboxylation of L-tyrosine by immobilized cells is linear for 20 min but reaching only 32% of conversion, then it practically stops. The time course of this L-aromatic amino acid decarboxylase activity shows the relative instability and the higher specificity of enzymes toward L-DOPA (Fig. 1). α-PNG and ß-PNG hydrolysis by immobilized cells is linear for 3.5 h, reaching 89% and 78% resp. of conversion, and then it practically stops.

Cells entrapped in beds (FURUYA et al. 1984) are cultivated in a similar way as the suspension cultures (temperature, pH, oxygen accessibility, etc.). On the other hand it was shown that the biotransformation ratio of cells entrapped in beds was correlated with the viability of the immobilized cells. The biotransformation ratio of co-

Table 3. Storage stability of immobilized cells

| Material | Relative activity (%) | | | |
|---|---|---|---|---|
| | DOPADC | TDC | α-GAL | β-GAL |
| Cell supension | 100 | 100 | 100 | 100 |
| Boiled cells | 0 | 0 | 0 | 0 |
| Fresh immobilized cells | 96 | 94 | 75 | 73 |
| Boiled immobilized cells | 0 | 0 | 0 | 0 |
| Immobilized cells after 1 month | 90 | 89 | 80 | 79 |
| Immobilized cells after 3 months | 85 | 84 | 92 | 91 |

deinone to codeine by fresh entrapped cells of *Papaver somniferum* was 70% and after 30 days of the function under optimal conditions the ratio decreased to 42%.

The storage stability of immobilized cells was tested by taking samples twice during three months, with a moderate loss of activity of all enzymes tested. We have found that in cells immobilized by glutaraldehyde the DOPADC, TDC, α-GAL and ß-GAL activities still reach high values (Table 3). The storage of the cells immobilized by glutaraldehyde in 0.15 mol/l NaCl with 0.02% sodium azide seems to be a very acceptable method for long-term preservation of the catalyst.

Like microbial cells, plant cells are able to biotransform various substrates mainly by these types of reactions: glycosylation, hydroxylation, reduction, esterification, epoxidation, isomerisation, oxidation, methylation (JONES, VELIKY 1981; FURUYA et al., 1984; TANG, SUGA 1994; HAMADA et al. 1994; TRINCONE et al. 1999; HAMILTON et al. 1984).

The immobilized cells have not been used on a large scale for industrial productions of secondary metabolites until now. However, their potential for fundamental scientific research is obvious (SZCZODRAC 1999; ANDRIAMAINTY et al. 2000). It is expected that after solving several problems (e.g. selection of stable high-producing strains), the immobilized cells will find their application in commercial production of high-value compounds. The multifunctional enzyme systems of viable and immobilized plant cells as well as their storage stability need further study.


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## Imobilizované rastlinné bunky v biotransformácii niektorých prekurzorov ópiových alkaloidov a glykozidov

**ABSTRAKT**: Bunky maku (*Papaver somniferum* L.) permeabilizované Tweenom 80 sa imobilizovali glutaraldehydom bez nosiča. Použitím takto imobilizovaných buniek maku siateho bola dokázaná dekarboxylácia L-tyrozínu a L-DOPA, pričom sa v priebehu troch mesiacov ich uchovávania nepozorovali preukazné zmeny dekarboxylázovej aktivity. Uvedeným spôsobom spracované bunky vykazovali aj vysokú α- a ß-galaktozidázovú aktivitu s dlhodobou stabilitou ako tiež výhodné mechanické vlastnosti. Dosiahnuté výsledky dokazujú možnosť uskutočnenia biotransformačných reakcií premeny prekurzorov ópiových alkaloidov, ale tiež niektorých glykozidov biotechnologickými technikami.

**Kľúčové slová**: imobilizácia buniek; L-DOPA a L-tyrozíndekarboxyláza; α- a ß-dekarboxyláza; α- a ß-galaktozidáza; *Papaver somniferum* L.

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# Chromosome number doubling in *Viola* × *wittrockiana* Gams. through colchicine treatment and its effect on early plant development

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**ABSTRACT**: Two methods of colchicine treatment were used to induce the doubling of chromosome number in two cultivars of *Viola* × *wittrockiana* Gams. – group of Pirna. The first method achieved by soaking or dipping swollen seeds in colchicine aqueous solution (0.1%, 0.3%, 0.5%), for three treatment periods (7, 14 and 21 hours). The second one by a treatment of the apex of young seedlings. One drop of colchicine aqueous solution (1%, 1.5%, 2%) was applied to the apex of the seedling every day during the treatment period (3, 4 and 5 days). The colchicine treatment has significantly influenced emergence and survival rates, plant morphology and growth rate. Root regeneration and morphological changes were investigated.

**Keywords**: *Viola* × *wittrockiana* Gams.; emergence rate; survival rate; colchicine; chromosome doubling; polyploidy; morphological changes; primary selection

Pansies (*Viola* × *wittrockiana* Gams.) are a popular perennial in cold climates, however they grow as an annual or biennial bedding plant (Smrž 1932). The intensive breeding programs, which are concentrated in Holland, Germany, USA and Japan, have led to many existing cultivars to select from for use in the landscape and for other ornamental purposes. The main aim of this study is to acquire new forms of higher ploidy level for subsequent breeding work.

The modern garden pansy is thought to have derived from a plenty of cross breeding – *V. altaica* (from Greece), *V. cornuta* (from the Pyrenees) and *V. lutea* and *V. tricolor* a native of Central Europe. According to Clausen (Emino, Sink 1968) Wittrock stated that the cultivated pansy was produced in the 1830s from a cross of *V. lutea* and *V. tricolor* (Novotná 1977; Horn 1956). According to Clausen, the chromosome number of *Viola* × *wittrockiana* Gams. 2n = 48 and the basic number $x = 6$. Emino, Sink (1968) reported the somatic chromosome number of all lines (*Viola* × *wittrockiana* Gams.) studied to be 2n = 48.

Demonstration of valuable characteristics in maximum value could be obtained in the case of optimal plant ploidy level (Uhlík et al. 1981). Plants with a double set chromosome number are generally larger or more robust than plants with the normal number of chromosomes. They may therefore be more profitable or have higher ornamental value. The high-level polyploidy plants usually have gigantic characteristics, such as thicker and wider leaves, deeper green color with larger stomata and larger flowers (Huang 1983 in Kafawin 1985). Polyploidy can be induced using chemicals such as colchicine. Normally, the spindle insures that either half of the set of chromosomes is reorganized into two new cells during cell division. Colchicine dissolves the spindle, so that the chromosomes remain in one cell (Nečásek, Cetl 1979; Uhlík et al. 1981; Laptev 1988; Abberton, Callow 1996).

Optimal conditions for colchicine treatment have to be established by experiments and for individual plant species (Laptev 1988). For better penetration of colchicine into the plant tissue, DMSO (dimethylsulfoxide, 2–4%) is recommended to be added to a colchicine solution (Brindza 1998).

Colchicine application to the apex of young seedlings has no effect on the roots, consequently it is better for plant vitality, but the efficiency of polyploidisation is a bit lower (Laptev 1988). A great problem of colchicine treatment of swollen seeds is the root regeneration as well as the root rot (Uhlík 1981; Laptev 1988).

## MATERIAL AND METHODS

As a starting material for the experiment, two cultivars of *Viola* × *wittrockiana* Gams. (Pure White and Light Blue) were chosen. The two cultivars belong to the Pirna

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group, which is characterized as frost-proof, early flowered with small or medium flower size and lower compactness.

For better colchicine penetration into the plant tissue, DMSO (dimethylsulfoxide, 3%) was added to a colchicine solution.

The treatment by the first method started under laboratory conditions (about 20°C). The seeds were placed in Petri dishes on a filter paper moistened with distilled water, for 24 hours. Then the swollen seeds were transferred and dipped in a colchicine solution in new dishes and they were kept in the dark for the treatment period. The experiment was repeated three times, each consisted of 9 variants for each cultivar. 300 germinable seeds were treated in each variant. The variants were established by combining treatment duration (7, 14 and 21 hours) with the concentrations of colchicine aqueous solution (0.1%, 0.3%, 0.5%). Against colchicine residual effects, the treated seeds were flushed with running water for three hours. Then the seeds were transferred to new dishes and placed on a new moistened filter paper to support root regeneration. Against rot appearance, the seeds were treated with Fundazole (0.1%) and then they were sown in flat trays with new substrate (white peat mixed with pearlite) and placed under the shade in the greenhouse (about 25°C/day and 18°C by night). The substrate was kept moistened until the seedling emergence, and then the seedlings were watered on demand. Chemical protection against pests and fungi was carried out 1–2 times per week. The seedlings were transplanted when they had the first 2–3 true leaves. The seedlings with morphological changes such as thicker, greener and smaller cotyledons and those with shortened and deformed hypocotyls were transplanted separately to support their survival because of their weakness and bad root regeneration. 20 seedlings with shortened hypocotyls were randomly selected and evaluated. Two months after the treatment, the seedlings were cultivated in the field, but the separated seedlings remained in the greenhouse and later they were transplanted into plant pots (ϕ 9 cm) and placed in a cold greenhouse. They were weak and unable to be cultivated in the field for wintering.

In order to detect doubled chromosome number plants, representative samples of plants were selected randomly and studied. The leaf index value (length/width) and the stomatal size were evaluated.

For the second method of colchicine treatment, untreated seeds were sown in multipots. 100 emerged seedlings were chosen for each variant. The experiment was repeated three times. Each replication consisted of 9 variants. The variants were established by combining treatment duration (3, 4 and 5 days) with the concentration of colchicine aqueous solution (1%, 1.5%, 2%). The multipots with young seedlings were placed in the greenhouse (about 25°C/day and 18°C/night). One small drop of colchicine solution was applied (with a dropper) to the apex between the cotyledon leaves immediately after their opening. The treatment was carried out once a day in the early morning hours while in the afternoon the seedlings were sprayed with water and they were treated 2× a week with fungicides such as Previcur 0.25%, Fundazole 0.25% and Rovral 0.1%. Because of the colchicine effect in the early plant development, the seedlings remained in the multipots for 82 days. Then they were transplanted in plant pots (ϕ 9 cm) and placed in a cold greenhouse for wintering. Leaf index (length/width) and stomatal size of randomly selected samples were evaluated as was mentioned in the first method.

## RESULTS AND DISCUSSION

### Emergence period

The colchicine treatment did not affect the emergence period. The seedlings from treated seeds and those from untreated seeds started their emergence at the same time (in 8 days from the sowing date).

### Emergence rate

The emergence rate was suppressed, mainly in the variants with longer treatment periods and higher colchicine concentrations. The treatment period was perhaps more effective than the colchicine concentration. The emergence rate was more suppressed by longer treatment pe-

Table 1. Colchicine treatment of swollen seeds, its effect on emergence rate (June 20 and August 30, 2000)

| | cv. Pure White | | cv. Light Blue | |
|---|---|---|---|---|
| Variant | No. of plants | Emergence rate (%) | No. of plants | Emergence rate (%) |
| Control plants | 300 | 100 | 297 | 99 |
| 0.1% 7 h. | 287 | 95 | 255 | 85 |
| 0.1% 14 h. | 254 | 84 | 220 | 73 |
| 0.1% 21 h | 249 | 83 | 160 | 53 |
| 0.3% 7 h. | 273 | 91 | 237 | 79 |
| 0.3% 14 h | 260 | 86 | 201 | 67 |
| 0.3% 21 h | 247 | 82 | 145 | 48 |
| 0.5% 7 h | 291 | 97 | 238 | 79 |
| 0.5% 14 h | 270 | 90 | 161 | 53 |
| 0.5% 21 h | 218 | 72 | 144 | 48 |

The basic number of treated seeds in each variant was 300 germinable seeds as was mentioned in the introduction

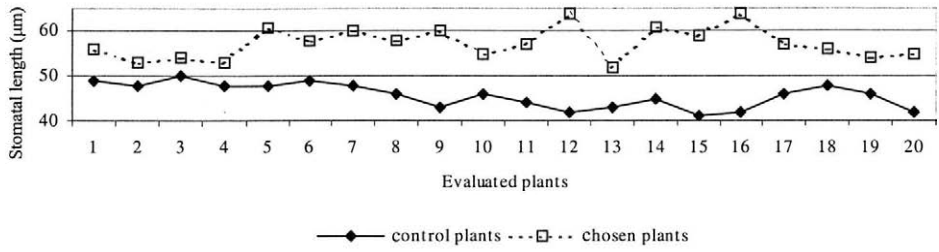


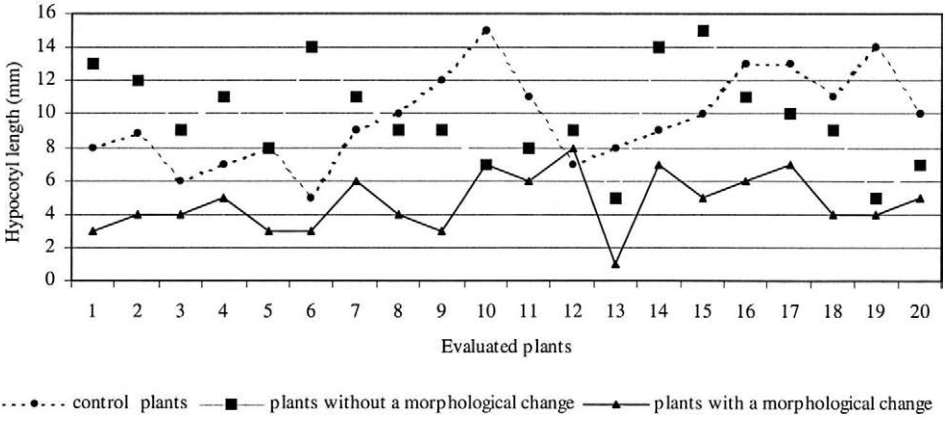

Fig. 1. Stomatal sizes comparison for a sample of chosen plants, *Viola* × *wittrockiana* Gams. – cv. Pure White, selected on the basis of leaf index value that was close to value 1

riod with lower colchicine concentration than by higher colchicine concentration with shorter treatment period. The emergence rate of the cv. Light Blue was more suppressed than that of the cv. Pure White (Table 1).

The sensitivity to the colchicine treatment was different and it depended on the cultivar. The cv. Light Blue showed more sensitivity than the cv. Pure White (Table 1). HANZELKA and KOBZA (2001) found similar results in *Callistephus chinensis* Nees.

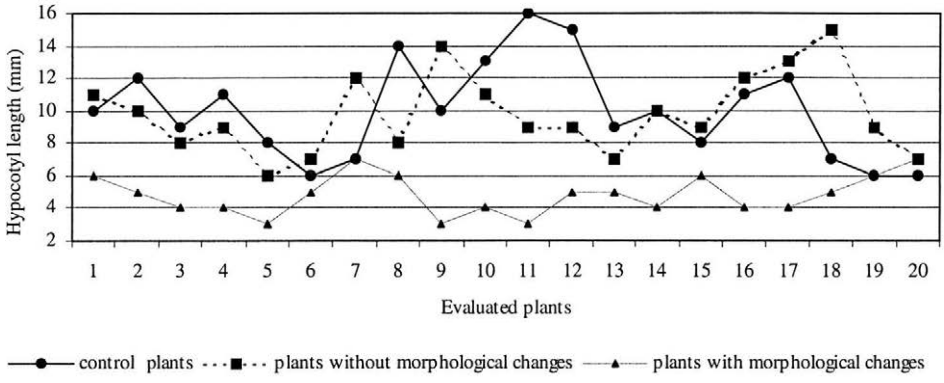
### Morphological changes

Colchicine treatment has caused a morphological change in the cotyledon leaves and hypocotyls of some young seedlings. Whereas the cotyledon leaves were thicker, harder, smaller and darker in color, the hypocotyls were shorter and thicker (Figs. 2 and 3). The development and growth of these seedlings were very slow compared to other seedlings in the same variants or with the control plants. They did not have enough regenerated roots or the roots were not developed, so many of them were unable to survive and they died (Figs. 4 and 5). The other seedlings from the treated seeds were similar to the control plants in all studied characteristics such as leaf index value (Fig. 6), stomatal size (Fig. 7) and growth rate. Most seedlings with morphological changes were acquired by longer treatment period and higher colchicine concentration (Fig. 8). The reason for these morphological changes might be the colchicine effect on the cell cytoskeleton and the presence of doubled chromosome number cells or mixoploid cells. According to primary selection, the treated seeds in this experiment have produced relatively low numbers of the expected plants. Their mortality rate was also very high and it was necessary to avoid root rot (Figs. 4 and 5). We have found that the colchicine treatment of the apex is easier and more efficient than the treatment of swollen seeds in *Viola* × *wittrockiana* Gams.




20 seedlings were randomly selected from each group (August 22, 2000)

Fig. 2. The effect of colchicine treatment of swollen seeds on the hypocotyl length (mm) of *Viola* × *wittrockiana* Gams. – cv. Pure White




For measuring, 20 seedlings were randomly selected from each group (August 22, 2000)

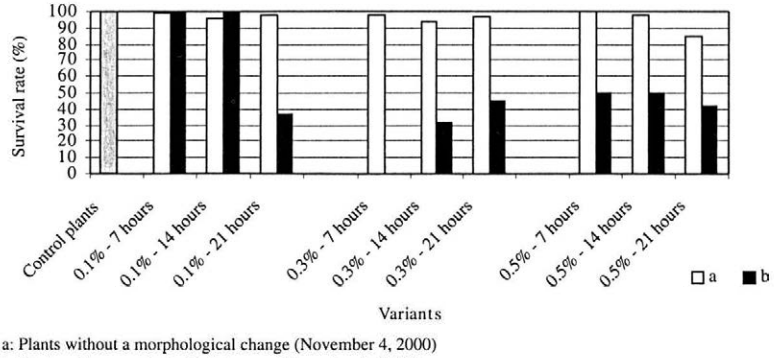
Fig. 3. The effect of colchicine treatment of swollen seeds on the hypocotyl length (mm) of *Viola* × *wittrockiana* Gams. – cv. Light Blue

In the second method (treatment of the apex), the growth and development of all treated seedlings were considerably inhibited for about two weeks. A lot of seedlings were damaged and died because of colchicine toxicity. The colchicine treatment considerably influenced the survival rate in all variants and mainly in the variants with longer treatment period and higher colchicine concentration (Fig. 9). The cotyledon leaves became thicker, harder and darker in color with silver luster. The surviving seedlings started their growth again after 2–3 weeks from the treatment starting date, but very slowly. The true leaves were also thicker with darker green color and the leaf shape became relatively rounded. The leaf index value (length/width) was smaller compared to control plants and too close to value 1 (Table 2). A similar result was obtained in the first method, but only in the plants with a morphological change.

The survival rate was hardly influenced by the second method. In the first method the survival rate of the plants with morphological changes was considerably influenced while in the other plants of the same variant no effect was found. In both methods, the survival rate was more influenced by the longer treatment period with higher colchicine concentration. Changes in plant morphology and stomatal size were important identifying factors for the primary selection as well as for the detection of new ploidy levels (Fig. 1).

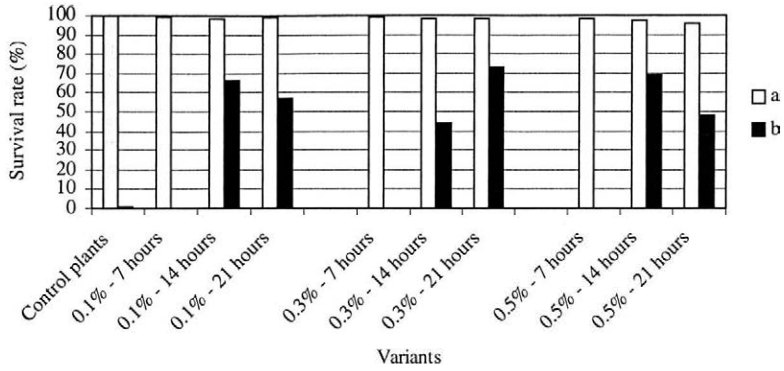
## CONCLUSION

The sensitivity to the colchicine treatment was different and it depended on the cultivar. The cv. Light Blue showed more sensitivity to the colchicine treatment than the cv. Pure White. The results of both treatment me-



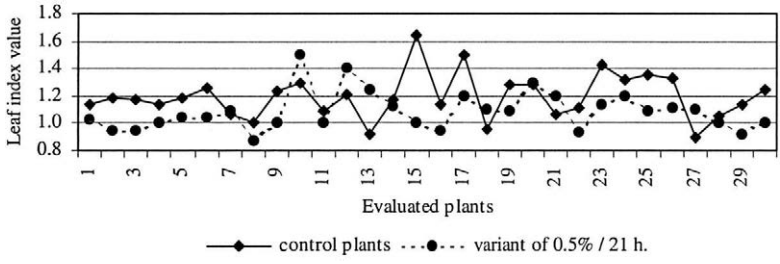

a: Plants without a morphological change (November 4, 2000)
b: Plants with a morphological change (November 4, 2000)

Fig. 4. The effect of colchicine treatment of swollen seeds on the survival rate of *Viola* × *wittrockiana* Gams. – cv. Pure White



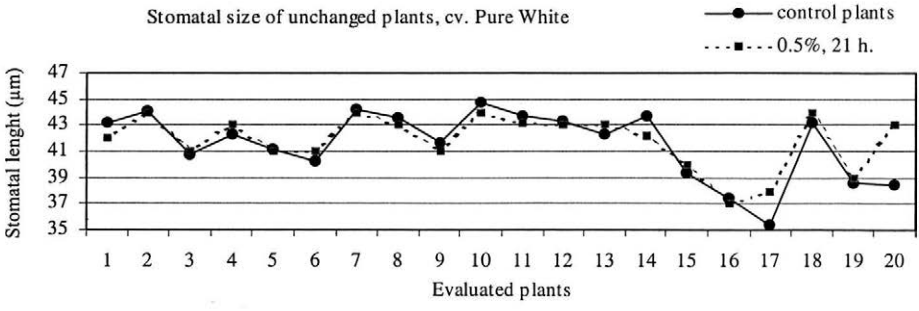

a: Plants without a morphological change (November 4, 2000)
b: Plants with a morphological change (November 4, 2000)

Fig. 5. Colchicine treatment of swollen seeds, its effect on the survival rate of *Viola* × *wittrockiana* Gams. – cv. Light Blue



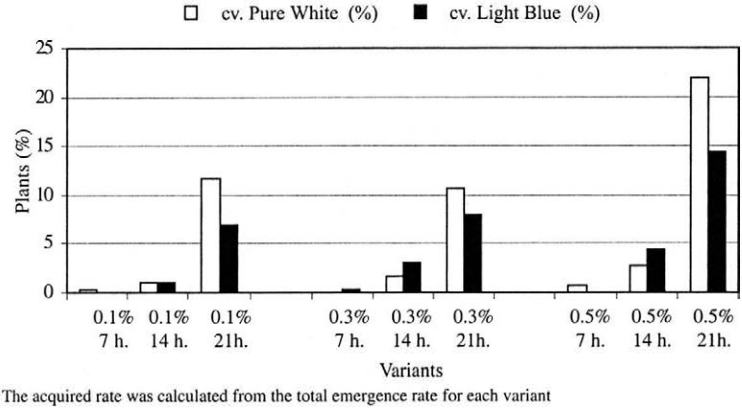

The evaluated plants did not include plants with a morphological change
The evaluated plants were from variants of the longest treatment period with the highest colchicine concentration

Fig. 6. Colchicine treatment of swollen seeds, cv. Pure White. The leaf index value of the plants that did not show a morphological change



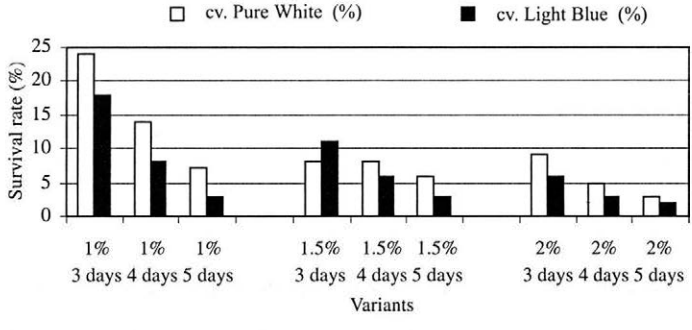

For measuring, 20 plants were selected randomly, but not from those with a morphological change (November 20, 2000)

Fig. 7. Stomatal size (μm) of plants from treated swollen seeds cv. Pure White, but without a morphological change




The acquired rate was calculated from the total emergence rate for each variant

Fig. 8. Colchicine treatment of swollen seeds, its effect on the emergence rate of plants with a morphological change (Jun. 20 and Aug. 30, 2000)




The basic number of treated seedlings in each variant was 100 seedlings
The survival rate of controlling plants was about 100%

Fig. 9. Colchicine treatment of the apex, its effect on survival rate

Table 2. Colchicine treatment of the apex, its effect on the leaf index value of *Viola* × *wittrockiana* Gams $M_1$ generation (November 10, 2000)

| cv. Light Blue | | cv. Pure White | |
|---|---|---|---|
| Variant | Leaf index value | Variant | Leaf index value |
| Control plants | 1.57 | Control plants | 1.41 |
| 1% – 3 days | 1.20 | 1% – 3 days | 1.04 |
| 1% – 4 days | 1.05 | 1% – 4 days | 1.07 |
| 1% – 5 days | 1.02 | 1% – 5 days | 0.91 |
| 1.5% – 3 days | 0.97 | 1.5% – 3 days | 0.98 |
| 1.5% – 4 days | 0.97 | 1.5% – 4 days | 1.10 |
| 1.5% – 5 days | 1.11 | 1.5% – 5 days | 1.20 |
| 2% – 3 days | 0.96 | 2% – 3 days | 1.08 |
| 2% – 4 days | 1.10 | 2% – 4 days | 1.10 |
| 2% – 5 days | 1.20 | 2% – 5 days | 1.10 |

thods correspond with other similar experiments and with the literature. In the case of colchicine treatment of swollen seeds, the toxic effect of colchicine led to a great problem in root regeneration and consequently to low emergence and survival rates. The colchicine treatment of the apex was easier and more effective and is therefore recommended.

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## Zmnožení počtu chromozomů u *Viola* × *wittrockiana* Gams. kolchicinem, vliv ošetření na raný vývoj rostlin


**ABSTRAKT**: Ke zmnožení počtu chromozomů pomocí kolchicinu u dvou kultivarů *Viola* × *wittrockiana* Gams. ze skupiny Pirnavské byly užity dvě metody. První metodou bylo namáčení nabobtnalých semen ve vodním roztoku kolchicinu s 0,1%, 0,3% a 0,5% koncentrací po dobu 7, 14 a 21 hodin. Druhá metoda spočívala v ošetření apexu mladých semenáčků kapkou vodního roztoku kolchicinu, nanášenou denně v 1%, 1,5% a 2% koncentracích po dobu tří, čtyř a pěti dnů. Roztok kolchicinu prokazatelně ovlivnil vzcházení a přežití semenáčků, morfologii a rychlost růstu rostlin. Byla také sledována regenerace kořenů a morfologické změny ošetřených rostlin.



**Klíčová slova**: *Viola* × *wittrockiana* Gams.; vzcházivost; míra přežití; kolchicin; zmnožení chromozomů; polyploidie; morfologické změny; primární selekce



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## AGROKOMPLEX 2001 V NITRE

Brány tohtoročného 28. Medzinárodného poľnohospodárskeho a potravinárskeho veľtrhu Agrokomplex 2001 v Nitre sa pre návštevníkov otvorili 16. Augusta 2001. Počet 543 zúčastnených vystavovateľov, ktorí sa prezentovali na celkovej čistej výstavnej ploche 35 000 m², mal prilákať nielen množstvo domácich, ale aj zahraničných návštevníkov. Tohtoročný podiel vystavovateľov zo zahraničia činil 22 % – 14 štátov.

Z hľadiska štruktúry vystavovateľov približne 35 % pripadlo na výrobcov a takmer 50 % tvorili obchodné firmy.

Agrokomplex je už 28 rokov podujatím naozaj jedinečným. Je miestom početných seminárov a konferencií, ktoré sú poznatkovou základňou pre všetky nové produkty spájajúce v jedno ekonomiku, vedu a techniku. Jeho úspešnosť každoročne závisí v značnej miere od schopnosti prispôsobovať sa v maximálnej možnej miere potrebám vystavovateľov, ako aj atraktívnosti sprievodných podujatí pre bežných návštevníkov. To sa organizátorom úspešne darí už viac ako štvrťstoročie. Výsledkom dlhodobého snaženia organizátorov je skutočnosť, že viac ako 80 % vystavovateľov prezentuje svoju firmu na veľtrhu opakovane; v roku 2000 to bolo dokonca 84 % vystavovateľov.

Návštevníci sú veľtrhu dokonca ešte vernejší. Každoročne veľtrh navštívi opakovane viac ako 90 % z nich. To, čo má ľuďom imponovať, musí mať aj ducha. Ani J. W. Goethe, ktorý túto vetu sformuloval, by určite neváhal označiť Agrokomplex za hmotný, ale aj duchovný podklad našich poľnohospodárov a potravinárov. Prezentácia alebo účasť na veľtrhu je akousi odmenou ich celoročného snaženia. Agrokomplex je jediný slovenský veľtrh, na ktorom sa môžu návštevníci stretnúť „zoči-voči" so živými rastlinami, ktorých kráľovstvom sú výstavné políčka. Neoddeliteľnou súčasťou tejto plochy bola aj spoločná expozícia Farmaceutickej fakulty UK a Slovakofarmy, a. s., Hlohovec. Sortiment vybraných druhov liečivých rastlín, ktorý slúži pre osvetové a pedagogické účely, komisia ocenila Čestným uznaním. Aj samotná veda v posledných rokoch uznáva, že nejeden domáci liečivý prostriedok pôsobí na ľudské telo lepšie, no predovšetkým jemnejšie ako „chemický zázrak" farmaceutického priemyslu.

Nie všetky domáce liečivá našich predkov by ale obstáli vo vedeckej skúške. Čím ďalej sa veda vyvíja, tým častejšie musia bádatelia pripustiť: niečo na tom prekvapujúcom pôsobení starých tradičných domácich prostriedkov predsa len bude. Dnes sa nemusíme uspokojovať len so zdedenými poznatkami našich predkov – už presne vieme, prečo sú bylinky a prípravky z nich takými cennými liečivami. Moderná veda analyzovala zloženie azda každej rastliny a preskúmala jej pôsobenie. Takto sa v posledných desaťročiach objavilo nespočetné množstvo látok – účinných i menej účinných, hojne zastúpených i takých, ktoré sa vyskytujú len v nepatrných množstvách. Postupne sa spresňovalo, ktoré rastliny majú na zdravie človeka liečivé účinky a ktoré sú skôr nevhodné.

Informácie o liečivých rastlinách, ich účinkoch, použití, ako aj o samotnom pestovaní zabezpečovala stála konzultačná služba. O scenár tejto spoločnej expozície sa zaslúžili RNDr. T. Lindauerová, CSc., a RNDr. J. Stano, CSc., za účinnej pomoci RNDr. A. Chocholatej, M. Jozefkovej, K. Maňúchovej, doc. DrPH. PhMr. J. Kresánka, CSc., J. Kvašňáka, Ing. P. Čupku, CSc., Ing. M. Koreňovej a Ing. Ľ. Procházkovej (garantky výstavy).

Okrem expozície liečivých rastlín bol pre návštevníkov výstavy pripravený prehľad výrobného sortimentu čajov, tekutých prípravkov, kapsulí a iných výrobkov firmy Slovakofarma, a. s. Výrobky tejto firmy prezentovali RNDr. A. Chocholatá, M. Jozefková a K. Maňúchová zo Slovakofarmy, a. s., divízia Liečivé rastliny Malacky, ktoré zároveň poskytovali návštevníkom aj vzorky rôznych druhov čajov.

Je potešiteľné, že z roka na rok sa zvyšuje záujem o pestovanie liečivých rastlín. Ľudia si čoraz viac uvedomujú liečivú silu prírody, využívajú liečivé rastliny, ktoré nám ponúka, a zároveň sa snažia plochy liečivých rastlín rozširovať.

Veľký počet návštevníkov aj tento rok potvrdil opodstatnenosť každoročnej spoločnej expozície, ktorá sa tešila záujmu nielen domácich, ale aj zahraničných návštevníkov. Veríme, že spoločná expozícia Farmaceutickej fakulty UK a Slovakofarmy, a. s., Hlohovec splnila svoj cieľ a obohatila širokú verejnosť o nové poznatky.

Zároveň si dovoľujeme zablahoželať všetkým vystavovateľom, ktorých výrobky, resp. expozície, boli ocenené, a zaželať im úspešný prienik výrobkov nielen na domácom, ale aj na zahraničnom trhu.

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