



Corvinus University of Budapest

STUDIES ON FERTILITY OF SWEET CHERRY CULTIVARS
BY TRADITIONAL AND MOLECULAR METHODS

Theses

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1. INTRODUCTION

Sweet cherries are generally self-incompatible, thus reliable yield can be expected only if cultivars in an orchard bloom simultaneously and cross-compatible. In the 1970's the first self-compatible cultivars appeared that are productive even without pollinators.

As the result of the Hungarian sweet cherry breeding, a range of cultivars now available providing fresh fruit for the market from the end of May until the end of July. In this set there are several candidates for national registration and EU patenting. These cultivars will soon enter fruit growing, therefore knowledge on their pollination and fertilisation is of great importance.

The information on fertility conditions of novel cultivars as well as of old ones is rather scarce. Therefore the aim of this work was to clarify fertility relationships among Hungarian cultivars by using traditional and molecular methods.

Self-fertility (self-compatibility) of most fruit species is gametophytic, genetically controlled by the so-called *S*-locus with a series of alleles (*S*-alleles). The cultivars bearing the same *S*-alleles are cross-incompatible and belong to the same incompatibility group. Due to the development of molecular techniques research methodology in incompatibility of fruit species have changed. Approaches in this field are based on investigations of the *S*-locus: genes expressed in the style (*S*-RNase gene) and the pollen (*F*-box gene), on protein and DNA level. These results are aimed at establishing new incompatibility groups. Generally speaking, traditional test-crosses have become scarce and results obtained by molecular methods are not always checked in practice in the field. Although among fruit species sweet cherry incompatibility is widely been studied, the *S*-genotype of the Hungarian cultivars have not been determined.

The topic of this thesis was recommended by Sándor Brózik as there was a lack of information on the compatibility of his new cultivars. Our studies have started in 1999 by traditional outdoor pollination experiments. However, our results were in several cases inconsistent. A part of the work was destroyed by spring frosts in 2001 and 2002. In the meanwhile, we have acquired new methods in fertility studies. First we have initiated pollen tube growth studies that allowed us to observe fertilisation on the tissue level. Later increasing knowledge on sweet cherry molecular genetics let us to turn towards new directions. Since DNA based methods in sweet cherry incompatibility were introduced to

Hungary, fertility of fruit species now can be studied on genetic level. This thesis contains the results obtained both by traditional and molecular methods.

The aims of the work:

1. Characterisation of fruit set of sweet cherry cultivars following open- and self-pollination (autogamy and geitonogamy) by field test-crosses.
2. Determination of the level of cross-compatibility of sweet cherry cultivar pairs in the field.
3. Specification of cultivar groups that can / can not be planted together, according to the results obtained above.
4. Introduction of molecular methods in incompatibility and its application to Hungarian sweet cherry cultivars.
5. Determination of *S*-genotypes of sweet cherry cultivars grown in Hungary by molecular methods.
6. Reconciliation of the results obtained by molecular technique with field test crosses data.
7. Investigation of *in vivo* and *in vitro* pollen tube growth in sweet cherry styles.

2. MATERIALS AND METHODS

2.1. Field test-crosses

The artificial pollination experiments were carried out at Elvira research farm of the Research Institute for Fruitgrowing and Ornamentals. During blooming time temperature data were recorded by the measuring instruments located at the research farm.

2.1.1. Self-compatibility studies

The experiments were performed in 1999 and 2001. The cultivars tested were: 'Sándor', 'Pál', IV-6/66, IV-6/240, 'Alex', 'Stella'.

The treatments were as follows:

- Open pollination: the flowers of the cultivars tested could be pollinated by any of the neighbouring cultivars, the pollen transfer was natural.
- Unassisted self-pollination (autogamy): the isolated flowers could be pollinated just by their own pollen, pollen transfer was not provided (gravitation only)
- Artificial self-pollination (geitonogamy): the isolated flowers were artificially pollinated with the pollen of the given cultivar.

Percentage of fruit set was calculated from the number of pollinated flowers and fully ripen fruits gained. The level of self-fertility / fruit set of the cultivars were assessed according to the following scale:

- **the level of self-fertility following autogamy (NYÉKI 1989):**

1 entirely self-sterile	0%
2 self-sterile	0,1-1%
3 partially self-fertile	1,1-10%
4 self-fertile	10,1-20%
5 highly self-fertile	>20%

- **fruit set following open pollination (BRÓZIK and NYÉKI 1980):**

1 low	<10%
2 medium	10,1-20%
3 high	20,1-30%
4 extremely high	>30%

The effect of the years, treatments and cultivars have been evaluated by analysis of variance. Interaction in fruit set after self- and open pollination was studied by monofactorial linear regression analysis.

2.1.2. Cross-compatibility studies

Artificial pollination experiments were performed to determine cross-compatibility in 27 cultivar pairs between 1999 and 2003. The cultivar combinations tested were:

Maternal parents:	Paternal parents:
'Aida'	'Sándor', IV-6/66, IV-6/240
'Rita'	'Sándor', IV-6/66, IV-6/240
'Vera'	'Sándor', IV-6/66, IV-6/240
'Carmen'	'Alex', 'Katalin', 'Linda', 'Stella', 'Sunburst'
'Germersdorfi 3'	'Alex', 'Katalin', 'Linda', 'Stella', 'Sunburst'
'Kavics'	'Alex', 'Carmen', 'Katalin', 'Linda', 'Sunburst'
'Margit'	'Alex', 'Katalin', 'Stella'

The cultivars within a pair were chosen so that their blooming time overlaps.

In 2003 the *S*-allele results determined by PCR analysis were checked by field test-crosses in 36 cultivar combinations. In the knowledge of the *S*-genotypes compatible, partially compatible and incompatible cultivar pairs were selected. The cultivars tested (each of them acted as maternal and paternal partners) were:

III-42/114 ('Carmen'), IV-6/240, H 264 ('Botond'), 'Germersdorfi 3', 'Hedelfingeni óriás', 'Katalin', 'Krupnoplodnaja', 'Linda', 'Margit', 'Rita', 'Van', 'Vera'.

Isolated flowers of the maternal parents were pollinated artificially with pollen collected from the flowers (balloon stage) of the paternal parents. Percentage of fruit set was calculated in each isolation bag from the number of pollinated flowers and fully ripen fruits gained. Data of 2-5 years were summed up by cultivar pairs. On the basis of fruit set results incompatible (fruit set: 0-5%) and economically good yielding combinations (fruit set >15%) were determined.

2.2. Pollen tube growth studies

2.2.1. Pollen tube growth *in vivo*

In vivo pollen tube growth was studied for three years (2000, 2001 and 2002). The cultivar pairs tested were: 'Margit' x 'Alex', 'Germersdorfi 3' x 'Alex'.

Isolated flowers of 'Margit' and 'Germersdorfi 3' were artificially pollinated with 'Alex' pollen. Flower samples were collected after 24, 48, 72, 96 and 120 hours of pollination. The flowers were fixed in 70% FPA solution. The pollen tubes were stained with aniline blue according to the method of PREIL (1970).

The styles were squashed ("Quetsch" or "squash" preparations) and pollen tube growth were studied by fluorescent microscopy. The number of germinated pollen grains on the stigma and number of pollen tubes in the upper, middle and lower third of the style were recorded. For pollen tube growth dynamic studies in each third of the style the percentage of flowers penetrated by pollen tube was calculated. Differences in pollen tube growth among maternal cultivars were studied by analysis of variance.

2.2.2. Pollen tube growth in vitro

In vitro pollen tube growth were examined in 2002, in the following cultivar combinations:

‘Vera’ (S_1S_3) x ‘Van’ (S_1S_3) - Incompatible combination according to *S*-genotypes

‘Alex’ (S_3S_3) x ‘Van’ (S_1S_3) - Partially compatible combination according to *S*-genotypes

Artificial pollination were carried out according to the method described by SCHMIDT and TIMMANN (1997) as follows:

The flowers of ‘Alex’ - as being a self-compatible cultivar - were emasculated. The flowers of ‘Vera’ and ‘Alex’ were inserted into 1% agar medium and kept in Petri dishes at room temperature for 24 hours. The flowers then were pollinated with ‘Van’ pollen. After 48 hours of pollination the flowers were handled and examined as described at *in vivo* pollen tube growth.

2.3. Incompatibility studies by DNA based methods

These experiments were carried out at the laboratory of East Malling Research in 2003.

The accessions analysed were growing in the experimental field of the Research Institute for Fruitgrowing and Ornamentals in Érd. Cultivars used as standards were provided by East Malling Research, their *S*-genotypes were already been published (SONNEVELD et al. 2001, 2003; TOBUTT et al. 2001) and represent all alleles so far described.

The accessions analysed are listed below:

‘Aida’, ‘Anita’, ‘Alex’, ‘Badacsonyi óriás’, ‘Botond’, ‘Carmen’, ‘Cherry Self Fertile 46’, ‘Germersdorfi 1’, ‘Germersdorfi 3’, ‘Hedelfingeni óriás’, ‘Katalin’, ‘Kavics’, ‘Krupnoplodnaja’, ‘Linda’, ‘Magyar porc’, ‘Margit’, ‘Münchebergi korai’, ‘Pál’,

‘Péter’, ‘Rita’, ‘Sándor’, ‘Solymári gömbölyű’, ‘Szomolyai fekete’, ‘Trusenszkaja 2’, ‘Tünde’, ‘Valerij Cskalov’, ‘Vera’, IV-5/5, IV-6/66, IV-6/240, IV-13/120.

The cultivars used as standards were: ‘Early Rivers’ (S_1S_2), ‘Victor’ (S_2S_3), ‘Lapins’ (S_1S_4), ‘Napoleon’ (S_3S_4), ‘Late Black Bigarreau’ (S_4S_5), ‘Colney’ (S_5S_6), ‘Charger’ (S_1S_7), ‘Inge’ (S_4S_9), ‘Orleans 171’ (S_7S_{11}), ‘Schneiders Späte Knorpelkirsche’ (S_3S_{12}), ‘Noble’ (S_6S_{13}), ‘Dikkeloen’ (S_5S_{14}).

DNA was extracted from dormant buds according to a miniprep version of the CTAB method (DOYLE and DOYLE 1987), as modified by SONNEVELD et al. (2001).

In our molecular analysis the *S*-RNase gene was investigated which is the stylar component of the *S*-locus responsible for incompatibility. There are two intron regions in the Rosaceous *S*-RNase (the so called 1st and 2nd intron) that show length polymorphism in accordance with *S*-alleles. With consensus primers flanking these intron regions the 1st and 2nd introns of the *S*-RNase gene were amplified in PCR reactions. The *S*-alleles were identified on the basis of the length of the 1st and 2nd intron.

The final concentrations in the reactions, the PCR cycles and the gel electrophoreses were performed according to the protocol described by SONNEVELD et al. (2003). The electrophoretic banding patterns of the 1st and 2nd intron analyses were scored using the PCR product sizes given by SONNEVELD et al. (2003).

The genotypes deduced after 1st and 2nd intron analyses were confirmed with allele-specific PCR analysis that was performed for all cultivars tested and alleles so far reported ($S_1, S_2, S_3, S_4, S_5, S_6, S_7, S_9, S_{10}, S_{12}, S_{13}, S_{14}, S_{16}$). The concentrations, PCR cycles and gel electrophoresis was in accordance with the protocol described by SONNEVELD et al. (2001 and 2003).

3. RESULTS

3.1. Self-compatibility studies

In both years studied, most accessions gave higher than 10% fruit set after unassisted and artificial pollination treatments. The only exception, IV-6/240, produced 0.0% fruit set in 1999 and 3.3% in 2001 after autogamy. Even in case of geitonogamy, 0.0% fruit set was observed in both years. Comparing temperature data and fruit set, it was shown that fruit set differences among cultivars can not be attributed to year effect.

According to the average fruit set data, all cultivars proved to be self-compatible, except IV-6/240. After unassisted self-pollination the highest average fruit set was produced by IV-6/66 (44.6%) and 'Stella' (26.0%) - scored as 5 - highly self-compatible. The cultivar 'Pál' showed the lowest ability for autogamy (12.2%).

In case of geitonogamy, all self-compatible cultivars did well. 'Sándor' showed the highest fruit set (71.7%), while the lowest results - if we ignore the self-incompatible IV-6/240 - was obtained by 'Pál' (45.5%).

For open pollination, the lowest average fruit set was observed in IV-6/240 (20.6%), among self-compatible cultivars it was 'Pál' (33.6%). In this treatment, the highest fruit set was produced by 'Alex' (50.5%).

Regarding fruit set, we found significant differences among the years studied as well as among treatments. The fruit set after unassisted self-pollination was significantly lower than of open- and artificial self-pollination. Geitonogamy showed the highest average fruit set (46.9%), followed by open pollination (39.5%) and autogamy (20.2%).

We have analysed whether there are any correlation between the fruit set following open- and self-pollination. According to the results of linear regression analysis the level of self-fertility does not affect considerably the fruit set obtained by open pollination. Comparing the R^2 values, geitonogamy resembles more to natural pollination than autogamy.

3.2. Cross-compatibility studies, association of cultivars

According to our test-crosses results, the combinations 'Vera' x IV-6/240, 'Germersdorfi 3' x 'Linda' and 'Margit' x 'Katalin' are cross-incompatible. These findings were later proved by DNA analysis as well. Additional incompatible cultivar pairs were identified by molecular methods later (**Table 1**). On the basis of fruit set results and

information on blooming time of the cultivars we have classified the cultivar combinations as follows:

- - Good yielding (fruit set >15%) combinations where blooming time of the members overlaps, recommended combinations in an orchard:
'Aida' x IV-6/66, 'Carmen' x 'Katalin', 'Germersdorfi 3' x 'Stella', 'Germersdorfi 3' x 'Katalin', 'Rita' x IV-6/66, 'Rita' x 'Sándor', 'Vera' x IV-6/66.
- Good yielding combinations but blooming time does not overlap, not recommended:
'Vera' x 'Sándor'
- Poor yielding or - on the basis of test-crosses - incompatible combinations:
'Carmen' x 'Alex', 'Germersdorfi 3' x 'Alex', 'Germersdorfi 3' x 'Linda', 'Germersdorfi 3' x 'Sunburst', 'Kavics' x 'Alex', 'Kavics' x 'Carmen', 'Kavics' x 'Linda', 'Kavics' x 'Sunburst', 'Margit' x 'Alex', 'Margit' x 'Katalin', 'Margit' x 'Stella'.
- Combinations where additional experiments are required: 'Aida' x IV-6/240, 'Aida' x 'Sándor', 'Carmen' x 'Linda', 'Carmen' x 'Sunburst', 'Kavics' x 'Katalin', 'Rita' x IV-6/240.

When testing the practical reliability of *S*-alleles in the field, fruit set results in test-crosses corresponded to compatibility relationships determined by *S*-genotypes. In presumably compatible combinations fruit set - usually far - exceeded 5%. In presumably incompatible combinations - in spite of favourable weather conditions for pollination - in most cases fruit set was 0% or did not exceed 5%.

3.3. Pollen tube growth studies

3.3.1. Pollen tube growth in vivo

The dynamics of pollen tube growth was studied in fully a compatible ('Margit' x 'Alex') and a partially compatible combination ('Germersdorfi 3' x 'Alex'). In either years the pollen tube growth of 'Germersdorfi 3' x 'Alex' did not follow the route that was expected in a compatible - partially compatible combination: as passing the time of pollination the percentage of flowers bearing pollen tubes in their lower section was not increased. However, it was the opposite in case of the fully compatible 'Margit' x 'Alex' combination.

The analysis of variance did not show no significant differences in the number of pollen tubes in either sections of the style between 'Germersdorfi 3' x 'Alex' and 'Margit' x 'Alex' combinations. Summing up the three years' data, one-two days after pollination there were more pollen tubes in the upper section of the style of 'Germersdorfi 3' flowers than of 'Margit', however, on the third-fifth days in the middle-lower sections 'Margit' borne more tubes than 'Germersdorfi 3'.

3.3.2. Pollen tube growth in vitro

In the partially compatible 'Alex' x 'Van' combination, pollen tubes penetrated the bottom section of the style in 50% of the flowers. On the contrary, in each flower of the incompatible 'Vera' x 'Van' combination pollen tubes reached the upper third section of the style only. The pollen tube end was often swollen and - after aniline-blue staining - intensively fluorescent, which indicate incompatibility.

3.4. Investigation of S-genotypes of Hungarian cultivars by PCR based method

After 1st and 2nd intron analyses, the fragment sizes of standard cultivars corresponded to individual alleles as described (SONNEVELD et al. 2003), thus on the basis of the patterns of standard cultivars the alleles of the cultivars tested could be identified. Results obtained by allele-specific primers confirmed the genotypes deduced by using consensus primers (**Table 1**). The cultivars then had been assigned to incompatibility groups. Cultivars in the same incompatibility group are cross-incompatible.

Table 1: The *S*-genotypes of sweet cherry cultivars determined by PCR analyses according to incompatibility groups

Cultivar	Consensus primers, preliminary genotype	Allele-specific primers, confirmation														Final results	
		<i>S</i> ₁	<i>S</i> ₂	<i>S</i> ₃	<i>S</i> ₄	<i>S</i> ₅	<i>S</i> ₆	<i>S</i> ₇	<i>S</i> ₉	<i>S</i> ₁₀	<i>S</i> ₁₂	<i>S</i> ₁₃	<i>S</i> ₁₄	<i>S</i> ₁₆	Geno-type	Incomp. group	
Vera	<i>S</i> ₁ <i>S</i> ₃	+		+											<i>S</i> ₁ <i>S</i> ₃	II	
IV-6/240	<i>S</i> ₁ <i>S</i> ₃	+		+											<i>S</i> ₁ <i>S</i> ₃		
13/120	<i>S</i> ₁ <i>S</i> ₃	+		+											<i>S</i> ₁ <i>S</i> ₃		
Münchebergi korai	<i>S</i> ₃ <i>S</i> ₄			+	+										<i>S</i> ₃ <i>S</i> ₄	III	
Solymári gömbölyű	<i>S</i> ₃ <i>S</i> ₄			+	+										<i>S</i> ₃ <i>S</i> ₄		
Hedelfingeni óriás	<i>S</i> ₃ <i>S</i> ₄			+	+										<i>S</i> ₃ <i>S</i> ₄		
Kavics	<i>S</i> ₃ <i>S</i> ₄			+	+										<i>S</i> ₃ <i>S</i> ₄		
Botond	<i>S</i> ₃ <i>S</i> ₄			+	+										<i>S</i> ₃ <i>S</i> ₄		
5/5	<i>S</i> ₄ <i>S</i> ₅				+	+									<i>S</i> ₄ <i>S</i> ₅	V	
III-42/114 (Carmen)	<i>S</i> ₄ <i>S</i> ₅				+	+									<i>S</i> ₄ <i>S</i> ₅		
IV-3/41 (Anita)	<i>S</i> ₃ <i>S</i> ₆			+			+								<i>S</i> ₃ <i>S</i> ₆	VI	
IV-13/51 (Tünde)	<i>S</i> ₃ <i>S</i> ₅			+		+									<i>S</i> ₃ <i>S</i> ₅	VII	
Szomolyai fekete	<i>S</i> ₂ <i>S</i> ₄ ? <i>S</i> ₄ <i>S</i> ₇ ?		+		+			-							<i>S</i> ₂ <i>S</i> ₄	XIII	
Trusenzkaja 2	<i>S</i> ₅ <i>S</i> ₆					+	+								<i>S</i> ₅ <i>S</i> ₆	XV	
Valerij Cskalov	<i>S</i> ₁ <i>S</i> ₉	+							+						<i>S</i> ₁ <i>S</i> ₉	XVIII	
Linda	<i>S</i> ₃ <i>S</i> ₁₂			+							+				<i>S</i> ₃ <i>S</i> ₁₂	XXII	
Germersdorfi 1	<i>S</i> ₃ <i>S</i> ₁₂			+							+				<i>S</i> ₃ <i>S</i> ₁₂		
Germersdorfi 3	<i>S</i> ₃ <i>S</i> ₁₂			+							+				<i>S</i> ₃ <i>S</i> ₁₂		
Badacsonyi óriás	<i>S</i> ₃ <i>S</i> ₁₂			+							+				<i>S</i> ₃ <i>S</i> ₁₂		
Magyar porc	<i>S</i> ₃ <i>S</i> ₁₂			+							+				<i>S</i> ₃ <i>S</i> ₁₂		
IV-13/20 (Aida)	<i>S</i> ₆ <i>S</i> ₁₂						+				+				<i>S</i> ₆ <i>S</i> ₁₂	XXIV	
Katalin	<i>S</i> ₄ <i>S</i> ₁₂				+						+				<i>S</i> ₄ <i>S</i> ₁₂	XXVII (new)	
Margit	<i>S</i> ₄ <i>S</i> ₁₂				+						+				<i>S</i> ₄ <i>S</i> ₁₂		
Krupnoplodnaja	<i>S</i> ₅ <i>S</i> ₉					+			+						<i>S</i> ₅ <i>S</i> ₉	O	
Rita	<i>S</i> ₅ <i>S</i> ₂ ? <i>S</i> ₅ <i>S</i> ₇ ? <i>S</i> ₅ <i>S</i> _{új} ?		-			+		-							<i>S</i> ₅ <i>S</i> ₂₂		
IV-6/5 (Péter)	<i>S</i> ₃ <i>S</i> ₄ '			+	+										<i>S</i> ₃ <i>S</i> ₄ '	SC	
IV-6/66	<i>S</i> ₃ <i>S</i> ₄ '			+	+										<i>S</i> ₃ <i>S</i> ₄ '		
IV-6/12 (Sándor)	<i>S</i> ₄ ' <i>S</i> ₉				+				+						<i>S</i> ₄ ' <i>S</i> ₉		
IV-6/39 (Pál)	<i>S</i> ₄ ' <i>S</i> ₉				+				+						<i>S</i> ₄ ' <i>S</i> ₉		
Alex	<i>S</i> ₃ <i>S</i> ₃ '			+											<i>S</i> ₃ <i>S</i> ₃ '		
Cherry Self Fertile 46	<i>S</i> ₁ <i>S</i> ₃ '	+		+											<i>S</i> ₁ <i>S</i> ₃ '		

3.5. New scientific results:

1. Self-compatibility of the cultivars ‘Sándor’, ‘Pál’, IV-6/66 and self-incompatibility of the cultivar IV-6/240 was proved by test-crosses.
2. By artificial cross-pollination experiments the cultivar pairs, those association in an orchard is recommended, have been determined (‘Aida’ x IV-6/66, ‘Carmen’ x ‘Katalin’, ‘Germersdorfi 3’ x ‘Stella’, ‘Germersdorfi 3’ x ‘Katalin’, ‘Rita’ x IV-6/66, ‘Rita’ x ‘Sándor’, ‘Vera’ x IV-6/66).
3. Differences between the pollen tube growth of compatible ‘Margit’ x ‘Alex’ and partially compatible ‘Germersdorfi 3’ x ‘Alex’ were demonstrated. In Hungary such pollen tube growth studies on sweet cherry were not performed before.
4. The *S*-genotype of 28 sweet cherry cultivars, responsible for incompatibility have been determined by molecular methods. The cultivars have been assigned to incompatibility groups. Thus cross-incompatible cultivar combinations among Hungarian sweet cherry cultivars could be specified.
 - A new incompatibility group, XXVII have been proposed that fits the existing cultivar table and includes the cultivars ‘Katalin’ and ‘Margit’.
 - In ‘Rita’ a new allele, S_{22} was discovered that was not reported in cultivated sweet cherry, but found in wild cherry.
 - Molecular analyses of *S*-alleles proved that the cultivars ‘Hedelfingeni óriás’ (S_3S_4) and ‘Valerij Cskalov’ (S_7S_9) are not identical with the cultivars grown under the same/similar names ‘Hedelfingen’ (S_3S_5), and ‘Valerij Chkalov’ (S_7S_6), respectively.
 - ‘Cherry Self Fertile 46’, a cultivar widely used as the source of self-compatibility in Hungarian sweet cherry breeding, has been identified and its *S*-genotype has been determined (S_7S_3').

4. CONCLUSION

Traditionally, self- and cross-incompatibility relationship of sweet cherry was studied with test-crosses in the field. However, these results are greatly affected by several factors - weather, growing site, tree condition and health status, pollen quality, etc. - thus fruit set results can be inconsistent. *S*-allele results obtained by PCR technique allow us to specify cross-incompatible cultivars that belong to the same incompatibility group. On the other hand, even some genetically compatible / partially compatible cultivar pairs can set poorly (e.g. the poor cropper ‘Germersdorfi óriás’ and its descendants), therefore to check fruit set in these combinations field test-crosses are still necessary. Our study on incompatibility aim at providing some information on cultivar association in orchards that growers and breeders can benefit in practice. However, for cultivar recommendations additional data - overlapping of blooming time, insect visitation - are needed.

The *S*-genotypes of ‘Germersdorfi 1’, ‘Germersdorfi 3’, ‘Badacsonyi óriás’ and ‘Magyar porc’ are identical and the same as of the German cultivar ‘Schneiders Späte Knorpelkirsche’ (S_3S_{12}). The relationship of these cultivars must be clarified by DNA fingerprinting (e.g. microsatellites). Similar situation occurs in case of ‘Hedelfingeni óriás’ (S_3S_4) / ‘Hedelfingen’ (S_3S_5), and ‘Valerij Cskalov’ (S_7S_9) / ‘Valery Chkalov’ (S_7S_6) cultivars. The above remind us the importance that in our experiments cultivar forms grown in Hungary must be tested and information on only true to name cultivars can be adopted.

On the evidence of its *S*-genotype and data published in the literature, the cultivar ‘Cherry Self Fertile 46’, a source of self-fertility in the Hungarian breeding programme is considered to be identical with the self-fertile JI 2538 selection from England.

We regard self-compatible cultivars as universal pollinators. However, there can be differences in their pollinating ability - probably due to variation in their pollen development, pollen quality or quantity - that must be considered when sorting the appropriate cultivars for new plantations.

From field test-crosses results we can conclude that fruit set of partially compatible cultivar pairs is practically not lower than of fully compatible ones.

Differences were found in pollen tube growth of ‘Margit’ x ‘Alex’ (fully compatible) and ‘Germersdorfi 3’ x ‘Alex’ (partially compatible). It is not clear whether the slow pollen tube growth in ‘Germersdorfi 3’ style could be attributed to its partial compatibility or it is related to ‘Germersdorfi 3’ to its own. In our opinion *in vitro* pollen tube growth is more

reliable for studying incompatibility than *in vivo* as in former case constant environmental conditions can be ensured. Castrating the anthers and petals (radical method) did not retard pollen tube growth.

In respect of breeding self-compatible sweet cherry cultivars DNA based *S*-genotyping is of special importance. Self-compatible seedlings in a certain progeny could be identified only among mature trees so far. At present by DNA based methods - with the knowledge of the genotype of their parents - they can be selected soon after germination. In the near future DNA analysis of the pollen component (F-box) gene will be useful for selecting self-compatible individuals and information on their parentage will not be necessary.

5. PUBLICATIONS RELATED TO THIS THESIS

Full papers published in journals (in English):

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