Incompatibility studies of Hungarian sweet cherry (*Prunus avium* L.) cultivars by traditional test crossings

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Summary: Cross-incompatibility is a common phenomenon between various sweet cherry (*Prunus avium* L.) cultivars. Traditionally, choosing cross-compatible cultivar pairs is based on test crossings in the field.

There is a lack of information about fertility relations of novel Hungarian sweet cherry cultivars and selections. We have studied cross-incompatibility in 42 sweet cherry cultivar pairs by test-crossings in the field. Out of those, 3 combinations showed incompatibility and 15 pairs were compatible.

Test-crossing results proved that with the knowledge of *S*-allele constitution of Hungarian cultivars incompatible cultivar pairs are recognised in practice reliably. However, we assume that in sterility not only the *S*-gene system, but other factors (e.g. abnormal development of pollen or flower) also occur, therefore, their examination would be needed.

Key words: incompatibility, sweet cherry, cross-pollination

Introduction

Most cultivars of sweet cherry are self-incompatible and various pairs of cultivars are cross-incompatible. Traditional test-crosses in the field are valid and a well-known method for determining cross-compatible cultivar combinations, which are important to sweet cherry growers and breeders. Since the beginning of 20th century incompatibility studies have been carried out in sweet cherry throughout the world (*Gardener,* 1914; *Kobel & Steinegger,* 1933). Incompatibility in sweet cherries is attributed to the multiallelic locus *S* (*Crane & Lawrence,* 1929). Cultivars bearing the same combination of *S*-alleles form an incompatibility group and are mutually cross-incompatible but compatible with all other cultivars belonging to other groups. In 1969 *Matthews & Dow* published their classic work describing the *S*-alleles responsible for compatibility of 160 cultivars on the basis of test-crosses. Naturally, breeders were interested in clarifying compatibility relationships among cultivars important in their own country. *Stösser* (1966) determined compatible and incompatible combinations among local German cultivars. *De Vries* (1968) established fertility groups with different levels of fruit sets and so described cross-incompatibility.

Schmidt & Timmann (1997) determined the *S*-genotypes of the cultivars originated from the research institute at Jork by test crosses and pollen tube growth studies, which was completed by *Stehr* (2000). *Godini* et al. (1998) compared the level of self- and cross-compatibility of some sweet cherry cultivars. *Wolfram* (1999) determined the level of self-fertility of some German sweet- and sour cherry hybrids.

In Hungary, *Maliga* (1952) tested the cross-compatibility of 'Badacsonyi óriás' and 'Germersdorfi óriás' with other Hungarian cultivars and determined two inter-incompatible cultivar pairs. Likewise, *Brózik* (1962) carried out compatibility studies in 'Germersdorfi óriás' and determined suitable pollinators. *Brózik & Nyéki* (1975) specified groups of Hungarian cultivars that are not only cross-compatible but bloom simultaneously. These authors later determined some incompatibility groups of Hungarian cultivars (*Brózik & Nyéki* 1980). *Nyéki* (1989) classified various Hungarian and foreign cultivars into incompatibility groups by means of field crosses, work completed by *Nyéki and Szabó* (1995). *Apostolné* (1994) clarified compatibility relationships of several new Hungarian cultivars. *Szabó* et al. (2002) summarised the knowledge about Hungarian sweet cherry compatibility.

Beside traditional methods, molecular techniques appeared that are useful in determining the *S*-alleles responsible for incompatibility in sweet cherry. These methods developed by *Boškovic & Tobutt* (1996) are generally used in several countries of the world. In this way the *S*-alleles of Hungarian cultivars have been identified. (*Békefi* et al., 2003).

The aim of this work was to specify cross-compatible and incompatible cultivar combinations important to Hungary by test crosses in the field. Furthermore, we were concerned whether field test crossing results correspond to *S*-allele genotypes obtained by molecular technique (*Békefi* et al., 2003) and the knowledge of *S*-alleles make choosing cross-compatible cultivar pairs easier in practice.

Material and method

Artificial test-crosses were carried out in the experimental field of the Research Institute for Fruitgrowing and Ornamentals at Érd.

Flower buds in balloon stage were isolated with parchment bags on branches facing to the four cardinal points at the height of 1–2 meters. Depending on the number of trees available we isolated flowers on 1–5 trees per maternal cultivar. In each cultivar combination, approx. 100 flowers were isolated (2–7 bags). Open flowers were removed beforehand.

For obtaining pollen at anthesis we collected flower buds in their balloon stage. In some cases – as there were considerable differences in the blooming time of partners – branches with closed white buds were collected and put into water at room temperature, overnight. Next day anthers from flowers in balloon stage were obtained and dried for 24 hours at room temperature protected against direct light. Next day, when the anthers opened we helped releasing the pollen grains with a glass pestle. The pollen was kept in +4 C° until use.

When the flowers opened in the bags and the pistils were ready to receive the pollen the artificial pollination took place. Open flowers were removed from the bag. Pollen grains were transferred to the stigma surface with the aid of wooden toothpicks. The pollinated flowers were counted and isolated again. Parchment bags were removed after petal fall. Fruit set was recorded at ripening time of each cultivar.

In each isolator bag and combination, percentage of fruit set was calculated. Data of 2–5 years were summed up for each combination.

Researchers have different opinion about the percentage of fruit set as a limit of cross-incompatibility. *Tehrani & Lay* (1991) regarded a combination compatible if fruit set exceeds 3%. In our evaluation we used the system below, developed by *Nyéki & Szabó* (1995) that can also be used in other stone fruit species:

	fruit set
incompatible combination:	0–1%
partially incompatible combination:	1.1–5%
compatible combination:	> 5%

Results

a) Determining cross-incompatible cultivar combinations by field test crosses

Fruit set data are shown in *Table 1.* A combination was regarded incompatible when its fruit set did not exceed 5% in any of the years studied. Consequently, the following combinations showed incompatibility:

IV-13/20 ('Aida') x IV-6/12 ('Sándor')
'Vera' x 'IV-6/240'
'Germersdorfi 3' x 'Alex'
'Germersdorfi 3' x 'Linda'
'Kavics' x 'Linda'
'Kavics' x 'Alex'
'Kavics' x III-42/114 ('Carmen')
'Linda' x 'Germersdorfi 3'
'Margit' x IV-6/39 ('Pál')
'Margit' x 'Katalin'

In 'Germersdorfi 3' x 'Alex' combination, fruit set was lower than 5%, in four years, however, in 2002 it was considerably high (34.6%). This high deviation is attributed to experimental error.

In some cases, the lack of fertilisation can not be attributed to the *S*-genes. For instance, in combinations, where 'Margit' or 'Kavics' appear as mother parents low fruit set may mean not only incompatibility, since these cultivars have poor results also in other combinations *(Table 1.)*. According to our observations, 'Margit' and 'Kavics' have low fruit set regularly, after open pollination even at various growing sites.

According to the literature, all self-fertile cultivars are universal pollen donors, they form compatible combinations with all other cultivars (*Thompson*, 1996). Pollen grains bearing self-fertility *S*-allele lost their activity in the incompatibility reactions therefore their pollen tube can reach the ovary without any difficulties. In cultivar combinations above IV-6/12 ('Sándor'), 'Alex' and IV-6/39 ('Pál') are proved to be self-fertile (*Brózik & Apostol*, 2000). Thus in those combinations, where they are the polliniser, poor fruit set can not be attributed to *S*-alleles.

In the above situations, the factors responsible for poor fruit set should be found elsewhere (weather conditions, morphological sterility, pollen quality, abnormal embryo development, etc.). Thus, undoubtedly cross-incompatible combinations are:

'Vera' x 'IV-6/240'
'Linda' x 'Germersdorfi 3'
'Germersdorfi 3' x 'Linda'

Among 42 combinations, 15 produced higher fruit set than 5% in the years tested, therefore we considered them compatible *(Table 1.)*. The highest fruit set was 78.3%, when 'Rita' was pollinated with 'IV-6/66' in 2000.

It was not always possible to ascertain compatibility relationships in some combinations as high variance among years was observed. In those cases the mother parent was often 'Margit' or 'Kavics', which have low yielding ability, generally.

b) Applicability of S-genotypes of Hungarian sweet cherry cultivars in practice

The *S*-genotypes of sweet cherry cultivars and selections important to Hungary have been determined recently (*Békefi* et al., 2003). In 2003, we carried out artificial pollination experiments in order to test their *S*-alleles in practice.

Table 1 Fruit set (%) of sweet cherry cultivars after cross-pollination (Érd-Elvira, 1997–2003) (combinations in *italics* are incompatible, **bold:** compatible)

Female	Year	Male: IV-6/12 (Sándor)	IV-6/240	IV-6/39 (Pál)	IV-6/66	IV-13/20 (Aida)	Katalin	Linda	Sunburst	Van	Alex	Stella	III-42/114 (Carmen)	Germersdorfi 3
IV-13/20 (Aida)	2000	3.3	**17.0**	24.2	24.0									
	2001	3.3	**8.9**	4.0	1.3									
	2003				34.3									
Vera	1997	**58.6**												
	2000	**24.2**	*0.0*		**78.3**									
	2001	**7.9**	*1.0*		**8.6**									
	2003		*0.0*											
IV-5/62 (Rita)	2000	**12.9**	**33.6**		**24.8**									
	2001	**17.5**	**15.8**		**17.8**	**29.7**								
	2003	**29.8**	**36.2**		**49.4**	**33.0**								
III-42/114 (Carmen)	2000			**9.0**							2.8	9.1		
	2001			**7.8**			**31.2**	0.0	**7.9**	**18.3**	7.3	0.0		
	2002			**23.1**							13.4			
	2003			**21.5**			**37.6**	35.2	**41.9**	**37.4**	6.7	19.3		
Germersdorfi 3	1997											**40.9**		
	1998					12.5	**23.3**	*0.0*	7.7		3.4		3.6	
	1999					1.8			0.8		4.6	**21.4**	16.2	
	2000										0.8			
	2001										1.4			
	2002										34.6			
	2003						**15.0**	*0.0*					9.8	
Katalin	1998								21.7					
	1999		**29.6**						3.2					
	2003		**58.5**											
Kavics	1998					2.1	0.0		0.0		0.0		0.0	
	1999					1.3			0.0	0.5	2.4		0.7	
	2001			4.0		7.7	15.7	0.8	9.7	6.6	2.5			
	2002			5.3		0.0	9.8	0.6	2.6	7.1	4.7			
Linda	1998								1.3				**16.5**	
	1999								6.3					*2.9*
	2003												**35.0**	*4.1*
Margit	1999						0.0				12.8	4.1		
	2000			5.0							9.7	5.3		
	2001			4.8							6.6	1.0		
	2002			2.7							3.5			
	2003						0.0							

Cultivars with known *S*-genotypes were chosen among which compatible, partially incompatible and incompatible combinations were formed. Crosses were made in both directions *(Table 2.)*.

In 2003, weather conditions were favourable for outdoor crossings, the temperature was at an optimum during the cherry flowering season. Generally, in presumably compatible combinations, fruit set was far higher than 5%. In spite of ideal weather conditions and a very productive year in most cases presumably incompatible combinations produced 0% or not higher than 5% fruit set.

'Linda' x 'Germersdorfi 3' had 4.1% fruit set and we also got fruit when 'Hedelfingeni óriás' was pollinated with 'Botond' and vice versa that was not expected from the *S*-genotypes. These phenomena are attributed to parthenocarpy.

In presumably partially incompatible combinations, fruit set was higher than 1% and even 5%.

Discussion

There are only few data available about the fertilisation of novel Hungarian cultivars, some of them were tested by *Apostolné* (1994). However, till now information about cross-incompatibility of the latest selections and cultivars were not at the disposal of growers, up to now.

According to our results referring to fruit set and *S*-alleles, the following cultivar combinations are incompatible:

'Katalin' x 'Margit'
'Germersdorfi 3' x 'Linda'
'Margit' x 'Katalin'
'Linda' x 'Germersdorfi 3'
'Van' x 'IV-6/240'
'Vera' x 'Van'
'IV-6/240' x 'Van'
'Van' x 'Vera'
'Botond' x 'Hedelfingeni óriás'
'Vera' x 'IV-6/240'
'Hedelfingeni óriás' x 'Botond'
'IV-6/240' x 'Vera'

Apostolné (1994) regarded 'Kavics' as a good polliniser for 'Hedelfingeni óriás'. Although we did not performe test crosses, as they both belong to the same incompatibility group III. (*Békefi* et al., 2003) they must be cross-incompatible with each other.

Table 2 Results of intentional field test crosses for controlling practical function of S-alleles of sweet cherry cultivars (Érd-Elvira, 2003)

female	*S*-genotype	male	*S*-genotype	fruit set (%)
Presumably compatible combinations:				
IV-5/62 (Rita)	S_5S_x	Vera	S_1S_3	17.5
Vera	S_1S_3	IV-5/62 (Rita)	S_5S_x	66.1
IV-5/62 (Rita)	S_5S_x	IV-6/240	S_1S_3	36.2
IV-6/240	S_1S_3	IV-5/62 (Rita)	S_5S_x	35.3
Vera	S_1S_3	Katalin	S_4S_{12}	60.9
Katalin	S_4S_{12}	Vera	S_1S_3	27.5
Katalin	S_4S_{12}	IV-6/240	S_1S_3	58.5
IV-6/240	S_1S_3	Katalin	S_4S_{12}	37.3
Katalin	S_4S_{12}	Krupnoplodnaja	S_5S_9	30.7
Krupnoplodnaja	S_5S_9	Katalin	S_4S_{12}	63.2
III-42/114 (Carmen)	S_4S_5	Germersdorfi 3	S_3S_{12}	37.9
Germersdorfi 3	S_3S_{12}	III-42/114 (Carmen)	S_4S_5	9.8
III-42/114 (Carmen)	S_4S_5	Linda	S_3S_{12}	35.2
Linda	S_3S_{12}	III-42/114 (Carmen)	S_4S_5	35.0
Botond	S_3S_4	Krupnoplodnaja	S_5S_9	32.7
Krupnoplodnaja	S_5S_9	Botond	S_3S_4	52.7
Hédelfingeni óriás	S_3S_4	Krupnoplodnaja	S_5S_9	9.2
Krupnoplodnaja	S_5S_9	Hédelfingeni óriás	S_3S_4	64.6
Krupnoplodnaja	S_5S_9	Margit	S_4S_{12}	54.4
Margit	S_4S_{12}	Krupnoplodnaja	S_5S_9	4.0
Presumably incompatible combinations:				
female	*S*-genotype	male	*S*-genotype	fruit set (%)
Katalin	S_4S_{12}	Margit	S_4S_{12}	0
Margit	S_4S_{12}	Katalin	S_4S_{12}	0
Van	S_1S_3	IV-6/240	S_1S_3	0
IV6/240	S_1S_3	Van	S_1S_3	0
Botond	S_3S_4	Hédelfingeni óriás	S_3S_4	1.6
Hedelfingeni óriás	S_3S_4	Botond	S_3S_4	2.6
Germersdorfi 3	S_3S_{12}	Linda	S_3S_{12}	0
Linda	S_3S_{12}	Germersdorfi 3	S_3S_{12}	4.1
Vera	S_1S_3	Van	S_1S_3	0
Van	S_1S_3	Vera	S_1S_3	0
Vera	S_1S_3	IV-6/240	S_1S_3	0
IV-6/240	S_1S_3	Vera	S_1S_3	0
Presumably partially incompatible combinations:				
female	*S*-genotype	male	*S*-genotype	fruit set (%)
Katalin	S_4S_{12}	Germersdorfi 3	S_3S_{12}	48.6
Germersdorfi 3	S_3S_{12}	Katalin	S_4S_{12}	15.0
III-42/114 (Carmen)	S_4S_5	Krupnoplodnaja	S_5S_9	24.0
Krupnoplodnaja	S_5S_9	III-42/114 (Carmen)	S_4S_5	37.4

When pollinating 'Germersdorfi 3' with 'Katalin' or 'Stella', we had good fruit set, similarly to the results of *Apostolné* (1994). She considered 'Kavics' x 'Linda' and 'Margit' x 'Stella' compatible, however, in these combinations we got poor fruit set. Thus further test crosses would be required.

On the basis of results, it was not possible to distinguish compatible and partially incompatible combinations that were presumed from *S*-genotypes.

Results of test crosses correlate with the *S*-allele scores obtained by molecular techniques. Thus it was proved that genotyping sweet cherry cultivars on (in)compatibility is of practical importance. *Schmidt* et al. (1999) states that after determining *S*-alleles with molecular technique there is no need of test crosses. Indeed, knowing the *S*-alleles, incompatible combinations are identified without test-crosses. However, in compatible and partially incompatible combinations, field crosses are required and examination of other factors (pollen quality and quantity, flower morphology, etc.) is needed as in certain cultivars they may affect fruit set (probably e.g. in cultivars 'Margit' and 'Kavics').

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