S-GENOTYPE DIVERSITY IN WILD CHERRY POPULATIONS IN THE CZECH REPUBLIC

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Wild cherry (*Prunus avium* L.) *S*-genotyping is aimed to uncover and thus make it possible to select appropriate genotypes applicable in establishing commercial plantations and advanced forest tree breeding activities. The general and long-term aim is to increase genetic gain in economically valuable traits while maintaining sufficient genetic variability (represented by diverse *S*-alleles in population). We genotyped 123 accessions from wild cherry growing areas in the Czech Republic using polymerase chain reaction based length polymorphisms detection of *S-RNase* and *SFB* genes. The studied plant material revealed 18 different *S*-haplotypes, 54 *S*-genotypes corresponded to 25 defined incompatibility groups of cultivated sweet cherry. Eighteen unique *S*-genotypes were designated to group '0' as a universal pollinator. Eleven new incompatibility groups were found out, of which four were cross-compatible with sweet cherry cultivars. The most frequent was a new incompatibility group $S_{14}S_{21}$ followed by the group $S_{12}S_{14}$. The haplotypes S_{14} (13%) and S_1 (10%) were the most frequent whereas S_{20} was less frequent in the wild populations of cherry. The present study of *S*-genotyping in the wild cherry population reveals the genetic diversity structure of natural populations and hopefully will help define the breeding strategy including more accurate planning activities such as the optimal seed design of orchards.

Prunus avium L., *S*-haplotype, incompatibility groups

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INTRODUCTION

Wild cherry (*Prunus avium* L.) is an important hardwood deciduous tree species from the family *Rosaceae*. Its distribution is typically scattered and coherent natural populations are rare (R u s s e l l, 2003). Categorized among the top rated European hardwood timbers (A v r a m i d o u et al., 2010), wild cherry is considered a valuable tree producing highly valuable veneer or wood for furniture industry. Moreover, this species has an important ecological role in forest ecosystems (S a n t i et al., 1998), promoting biodiversity and sustainability within forest stands (S t o j e c o v á, K u p k a, 2009).

Cherries exhibit gametophytic self-incompatibility (SI), which is a common genetic mechanism promoting out-crossing in flowering plants. The SI is genetically controlled by multi-allelic gametophytic locus *S*, which

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encodes two linked genes – *S-RNase* and *SFB* (Crane, Lawrence, 1929; Bošković, Tobutt, 1996; Tao et al., 1999; Yamane, 2003). Genotypes bearing the same allele are cross-incompatible or considered as self-pollen incompatible (Kao, Tsukamoto, 2004; Takayama, Isogai, 2005; Zhang et al., 2009). Cultivars bearing the same alleles cannot pollinate each other and for practical reasons are placed in the same incompatibility group (IG), while genetically unrelated pollen are able to complete fertilization and are cross-pollen compatible. The conserved (C1–C3, RC4, and C5) and hypervariable regions (RHV) of *S-RNase* gene play an important role in discriminating between self and non-self-pollen in *P. avium* (Ushijima et al. 1998). The cultivar, which bears the unique combination of alleles and is able to perform fertilization with all of the known IGs, is called a universal donor and is placed in the distinctive group termed as '0' (Schuster et al., 2007). The phenomenon of gametophytic self-incompatibility (GSI) leads to a restricted number of potential pollen donors/recipients (Stanys et al., 2008). The SI analysis has largely been restricted to sweet cherries (Sharma et al., 2014, 2016), while no study has been conducted so far to assess the genetic diversity across wild cherry in the Czech Republic.

Foresters became conscious of wild cherry beneficial effect in forest stands (Jarni et al., 2012) and in the past century emphasized growing potentials of wild cherry. In the Czech Republic establishment of the seed orchard and progeny testing started in the 1990s (Kobliha, 2002), nevertheless tools of molecular biology have not been applied to breeding activities until now. The *S*-locus analysis is a crucial step for proposing effective breeding activities, such as designs of appropriate schemes of progeny trials and spatial scheme of seed orchards, enhancing efficiency of pollination processes.

Our research hypothesized differences in genetic variation among populations of diverse developmental processes. Hence we compared the *S*-locus analysis results of three *Prunus avium* natural and pseudo-natural protected populations, represented by selected trees in areas near the capital city of Prague (area of highly disturbed population) and Central and West Bohemian regions.

MATERIAL AND METHODS

One hundred twenty-three wild cherry trees from diverse locations in the Czech Republic were studied. The sampled population was represented by individuals from three geographically distinctive areas: (1) 53 wild cherry genotypes from the northwestern suburban area of the capital city of Prague where a substantial influence of sweet cherry cultivars as pollinators is expected (PWC), (2) 50 genotypes of wild cherry plus trees selected in the protected landscape area Křivoklátsko in the Central Bohemian region (CL), and (3) 20 genotypes of wild cherry plus trees originating from the military area Doupov in West Bohemia (CD) (Fig. 1). Populations from the Central and West Bohemian regions are expected to be natural and pseudo-natural with a low level of gene flow from sweet cherry cultivars.

Young leaves for DNA extraction were collected during the spring season. Genomic DNA was extracted from fresh young leaves following the instruction of DNeasy Plant Mini Kit (Qiagen, Germany). The *S*-locus analysis was performed by the polymerase chain reaction (PCR) using the primer pairs FBOX50A/F-BOX intronR for *SFB* genotyping by Vaughan et al. (2006) and PaConsI-F/PaConsIR2 for *S-RNase* genotyping by Sonneveld et al. (2003, 2006). Forward primers were fluorescently labelled with NED for *SFB* and 6-FAM for *S-RNase*. The PCR reactions with primers PaConsI-F/PaConsIR2 were carried out according to Wünsch, Hormaza (2004). The PCR reactions with primers F-BOX50A/F-BOX intronR were carried out as by Cachi, Wünsch (2014). PCR fragments were detected using an ABI PRISM 310 genetic analyzer and Peak Scanner v1.0 software, a standard 600-GeneScan LIZ was used for sizing (all Applied Biosystems, USA).

Eight sweet cherry cultivars of known *S*-genotype ('Aranka' S_1S_3; 'Belise Bodel' S_1S_9; 'Granát' S_3S_6; 'Moser' S_6S_{13}; 'New York 242' S_1S_{12}; 'Nočka II' S_5S_{13}; 'Ptačka z Plzně' S_5S_{16}, and 'Tim' S_4S_5 (Sharma et al., 2016) were used as *S*-allele size standards.

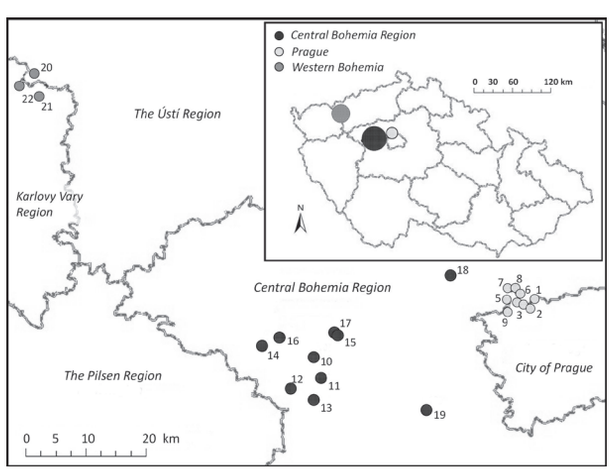

Fig. 1. Geographical location of wild cherry populations
Each location is represented by different shade of grey. The size of the circle in the cut out upper right corresponded with the area if sampling.

Table 2. Characterization of detected S-alleles (bp)

S-Allele	*S-RNase* (first intron)	*SFB* (5′UTR intron)
S_1	379	188
S_2	342	NA*
S_3	232	200
S_4	449	187
S_5	394	187
S_6	442	178
S_7	342	178
S_{10}	364	175
S_{12}	344	183
S_{14}	330	188
S_{16}	412	175
S_{18}	339	181
S_{19}	425	188
S_{20}	324	169
S_{21}	374	191
S_{22}	420	172
S_{28}	367	183
S_{31}	207	182

*NA: Not amplified

Length of amplification product (bp) obtained using fluorescently labelled S-RNase first intron consensus primers (VIC-PaConsI-F and PaConsI-R2) and fluorescently labelled SFB intron consensus primers (6-FAM-F-BOX5′A/ F-BOX intron-R) for a range of *P. avium* S - haplotypes, amplicons (ABI310) were compared to LIZ 600 standard.

RESULTS

General S-haplotypes diversity

None of the 123 analyzed genotypes appeared to be triploid. Totally 18 different standard *S*-haplotypes were identified in the following frequencies: S_1 (10%), S_2 (8%), S_3 (7%), S_4 (5%), S_5 (4%), S_6 (11%), S_7 (6%), S_{10} (2%), S_{12} (8%), S_{14} (13%), S_{16} (8%), S_{18} (2%), S_{19} (3%), S_{20} (less than 1%), S_{21} (5%), S_{22} (6%), S_{28} (1%), and S_{31} (1%). Frequency of the specific haplotype varied among geographical populations (Fig. 2). Totally 54 *S*-genotypes corresponding to 25 defined IGs for sweet cherry, 4 new IGs cross compatible to sweet cherry cultivars of group '0', and 11 new IGs were investigated (Fig. 3). Totally 20 different *S*-haplotypes were identified in the collections. In the eleven new IGs, alleles S_{14} and S_{12} were the most frequent, whereas S_7 allele was less frequent.

Size of the *S-RNase* amplification products determined by the ABI PRISM 310 Genetic Analyzer ranged from 207 to 449 bp. Sizes of the *SFB* amplicons ranged from 169 to 200 bp. Some *SFB* alleles (e.g. *SFB*4 and *SFB*5) showed very similar sizes (187 bp) to the S_1, S_{14}, and S_{19} alleles (188 bp) (Table 1). This small difference made their identification difficult. In such cases, *S*-haplotype was distinguished based on the differences in *S-RNase* amplicon. Here we noticed differences in amplicons sizing 2–3 bp when LIZ 600 or LIZ 500 standards were used.

Putative new *S*-haplotype identified

In two individuals from West Bohemia, a putative new haplotype was detected. In the present study it was labelled S_n until the subsequent confirmation by sequencing. The genotype CD 22 showed two strong peaks corresponding to S_{12} haplotype (344 bp for *S-RNase* and 183 bp for *SFB*) and one extra-peak (422 bp) representative for *S-RNase* which did not correspond to any known *S*-haplotype. Similarly, the CD 26 genotype showed strong peaks of sizes 374 bp (*S-RNase*) and 191 bp (*SFB*) representative for S_{21} haplotype and again one more unidentified 422 bp peak (*S-RNase*) similarly in previous observation. None different *F-box* specific fragment has been amplified in both samples (Table 1).

Assignment of new incompatibility groups

The *S*-genotyped accessions were assigned to twenty-five IGs of sweet cherry cultivars according

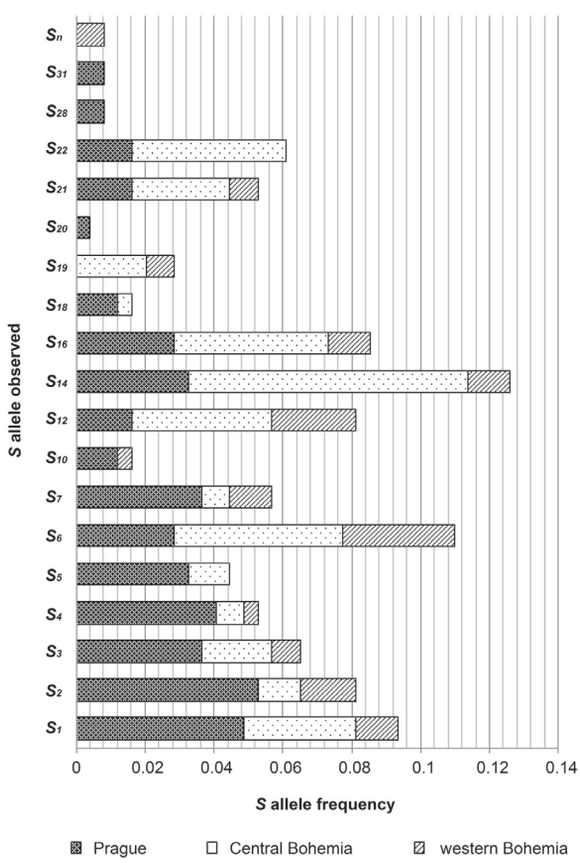

Fig. 2. Proportional and total frequencies of detected S-alleles

to Schuster et al. (2007) and eleven new IGs were defined (Fig. 3). In total 18 genotypes (12%) were assigned to '0' IG and should be under consideration for the potential extension of the harmonization table.

DISCUSSION

The first substantial study of incompatibility in wild cherry was done by Berger (1963). Other analyses of allelic diversity were implemented in Belgium and the UK (De Cuyper et al., 2005; Vaughan et al., 2008). Some particular alleles (S_{17}, S_{19}, $S_{21/25}$, and S_{22}) were also detected in Sicilian, German, and Hungarian commercial cultivars (Marchese et al., 2007; Schuster et al., 2007).

In the current study of 123 individuals, 18 different *S*-haplotypes were corresponding to 54 *S*-genotypes. Variation in *S*-alleles of the studied wild cherry population was significantly higher than reported in other studies (Bošković et al., 2005; De Cuyper et al., 2005). When comparing differences between the populations, 17 haplotypes from 53 trees sampled around Prague, 14 haplotypes from 50 trees in Central Bohemia, and 12 haplotypes within the population of 20 accessions in West Bohemia were differentiated. Taking that relatively, this indicates the highest genetic variation in the West Bohemian population, whilst a similar level of variability was found in Prague and the Central Bohemian Region. Therefore the West Bohemian population seems to be very contributive for the maintaining program of autochthonous wild cherry germplasms.

The haplotype with the highest frequency in our data set (S_{14} – 13%) also occurs in commercial cherry cultivars in West and Central Asia (namely in Iran; about 4%) and rarely in Europe (Germany, Italy, France, UK; up to 2%) (Schuster, 2012). Interestingly, that haplotype has never been detected among the Czech sweet cherry cultivars. In the wild collection it is the most frequent and thus can be considered as autochthonous for Czech wild cherry populations. The S_{16} haplotype is present across the collection (8% frequency) (Fig. 2). This haplotype has been reported as highly frequent in cherries from the Balearic Islands and from Sicily (Marchese et al., 2007) being found both in wild and cultivated cherries in North Europe (Sonneveld et al., 2003; Tobutt et al., 2004; De Cuyper et al., 2005; Stanys et al., 2008). Probably it is also autochthonous in the wild cherry populations, as the previous study (Sharma et al., 2016) did not confirm its occurrence among sweet cherries in the Czech Republic.

Opposite to our findings, De Cuyper et al. (2005) did not detect alleles S_4 and S_5 in wild cherry from Belgium. From its frequency being the highest in the suburban area it seems to be highly probable that S_4 and S_5 are not autochthonous in the Czech Republic and that they were secondarily introduced through cultivated cherries. A relatively low frequency of S_{10} in all regions of the Czech Republic indicates its marginal occurrence. Alleles S_{14} and S_{22} are very frequent in the Central Bohemian population, but appear to be less frequent or completely absent in West Bohemia. Newly detected alleles (S_n) were found in West Bohemia. The highest haplotype frequencies were observed for S_{14}, S_6, and S_1. Only rare presence of S_{20} was notable in our set of genotypes. The common occurrence of the allele is noticed in western Europe, namely in really distant Belgian wild cherry populations (De Cuyper et al., 2005).

The system of incompatible groups is of high importance mainly in a breeding strategy of sweet cherries. We are in congruence with other investigators (e.g. De Cuyper et al., 2005; Schuster, 2012) that it might be inappropriate to introduce many wild cherry accessions into the standard sweet cherry harmonization table. But there is a question whether the breeding populations of wild cherry would not require similar classification in order to increase the efficiency of forest tree breeding activities. As an acceptable alternative we suggest the traditional scheme of sweet cherry IG sorting established as an independent parallel system for wild cherry germplasm collections.

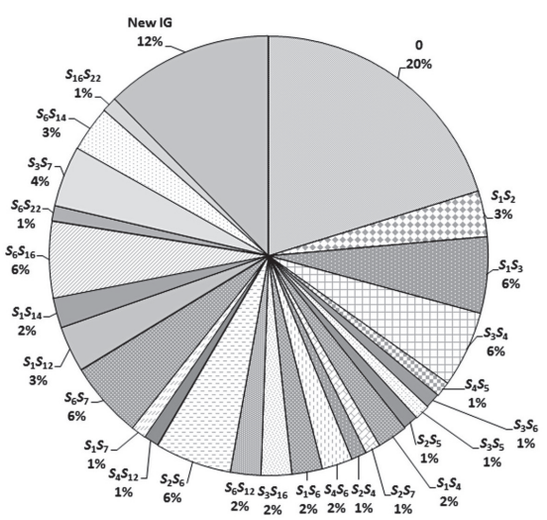

Fig. 3. Proportional representation of detected incompatibility groups (IGs)

CONCLUSION

Our paper presented the first systematic molecular study of wild cherry variability in the Czech Republic. A study and/or project of this kind have not been conducted in the Czech Republic despite an immense inevitability to describe pollination ability and status

of variability in natural condition and germplasm collections towards aiming the long term objectives.

The evaluation showed that some *S*-haplotypes were exclusive of a particular geographic region. A significantly higher frequency of haplotypes S_1 to S_6 was detected in the collection originating from the Prague surroundings, where the probability of gene-flow across local wild germplasm and cultivars of sweet cherries is higher. This presumption is confirmed by the frequent presence of S_{14}, S_{16}, and S_{22} haplotypes typical for wild germplasm. Namely the alleles S_4 and S_5 were notably present in the Prague region and less frequent in the Central Bohemian region, while S_5 was found to be absent in the West Bohemian region.

We observed and estimated an interesting and key phenomenon in natural population in protected areas of Central Bohemia. Some trees of identical SI group, originating from close geographic proximity, i.e. from the same forest stand, were investigated using a set of 12 microsatellite loci.

The members of the mentioned groups were proved to have an identical microsatellite genotype. It might be surprising; nevertheless in wild cherry the phenomenon of a high rate of vegetative reproduction has frequently been reported (e.g. Ducci, Santi, 1997; Schueler et al., 2006). If the preference of clonal self-propagation is frequent, it can dramatically change pollination rates in natural populations and lead to a decrease in natural variability.

We believe our study extended the knowledge of wild cherry *S*-locus variation in the Czech Republic and the results or methodology in general could be used as a tool for advanced forestry breeding activities.

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