The apple skin colour plays an important role in apple fruit trading, because customers prefer red coloured apple skin and thus the cost-effectiveness of red skin apples is greater. Molecular markers using enable to recognize the future fruit colour by creating a variety at the beginning of the breeding process. Fruit skin colour is determined by carotenoids, chlorophyll and anthocyanins (Lancaster 1992). The red colour is conditioned by anthocyanins, green and yellow colour is controlled by the quantity and the ratio of carotenoids and chlorophyll. There were three hypotheses about fruit skin colour control – single dominant gene control (Crane and Lawrence 1933), modifier genes overriding Rf locus (Schmidt 1988) and two complementary dominant genes (White and Lespinasse 1986). Cheng et al. (1996) confirmed that the skin colour of the apple (red/yellow dimorphism) is directed by a single gene localised in Rf locus and the presence of red anthocyanin pigmentation is dominant. By means of bulked segregant analysis and testing oligomer primer in crossing populations Cheng et al. (1996) detected four fragments associating with apple skin colour. Two of them – marked as A1 (1160 bp) and A2 (1180 bp) associate with red skin colour and a1 (1230 bp) and a2 (1320 bp) associate with yellow skin colour. These fragments were partly sequenced and there were found a high sequence of homologies confirming that fragments were generated from the same locus, Rf locus.

The incompatibility is the most widespread system preventing the pollination by its own or relative pollen. The incompatibility was studied in many plant families – Solanaceae, Brassicaceae, Rosaceae. The single locus gametophytic incompatibility is typical for the Malus species. The incompatibility is directed by a group of S-alleles localized at multialellic locus – the S-locus in the pistil. The S-alleles encode high basic proteins – glycoproteins with ribonuclease activity (McClure et al. 1989), thus they are often called S-RNases. These RNases specifically interact with similar S-locus in the male partner (Golz et al. 2001) – the pollen – and the recognition of relative or unrelated S-alleles in pollen direct the pollination and the fertilization. The pollination and the pollen-tube growth are inhibited, when the pollen and the pistil have the same S-allele. An S-alleles

**ABSTRACT**

The skin colour of 21 varieties was assessed on a molecular level. Four varieties had yellow skin colour with the genotype constitution a1a1, a1a2 or a2a2. Seventeen varieties had dominant red colour. Homozygous A1A1 constitution was present in 7 varieties, and heterozygous constitution A1a1 or A1a2 was found in the rest of the assessed varieties. The S-alleles controlled the incompatibility system of the pistil. Their detection is possible by means of the PCR method on the basis of allele specific primers. Six S-alleles (S1, S2, S3, S5, S7 and S9) were studied in the same collection of 21 diploid Czech varieties. This paper brings new findings on S-allele characterization, because the Czech varieties have not yet been studied on a molecular level. Both types of S-allele were found in 12 varieties. Only one type of S-allele was described in 9 varieties. Simultaneously, the presence of the Vf gene was screened in the collection of 21 Czech apple varieties. Ten varieties with a field resistance against the scab had a heterozygous constitution of the Vf gene. All 11 susceptible varieties were recessive homozygous vfvf.

**Keywords:** Malus; PCR; Vf gene; incompatibility; S-allele; skin colour; A-allele

The apple skin colour plays an important role in apple fruit trading, because customers prefer red coloured apple skin and thus the cost-effectiveness of red skin apples is greater. Molecular markers using enable to recognize the future fruit colour by creating a variety at the beginning of the breeding process. Fruit skin colour is determined by carotenoids, chlorophyll and anthocyanins (Lancaster 1992). The red colour is conditioned by anthocyanins, green and yellow colour is controlled by the quantity and the ratio of carotenoids and chlorophyll. There were three hypotheses about fruit skin colour control – single dominant gene control (Crane and Lawrence 1933), modifier genes overriding Rf locus (Schmidt 1988) and two complementary dominant genes (White and Lespinasse 1986). Cheng et al. (1996) confirmed that the skin colour of the apple (red/yellow dimorphism) is directed by a single gene localised in Rf locus and the presence of red anthocyanin pigmentation is dominant. By means of bulked segregant analysis and testing oligomer primer in crossing populations Cheng et al. (1996) detected four fragments associating with apple skin colour. Two of them – marked as A1 (1160 bp) and A2 (1180 bp) associate with red skin colour and a1 (1230 bp) and a2 (1320 bp) associate with yellow skin colour. These fragments were partly sequenced and there were found a high sequence of homologies confirming that fragments were generated from the same locus, Rf locus.

The incompatibility is the most widespread system preventing the pollination by its own or relative pollen. The incompatibility was studied in many plant families – Solanaceae, Brassicaceae, Rosaceae. The single locus gametophytic incompatibility is typical for the Malus species. The incompatibility is directed by a group of S-alleles localized at multialellic locus – the S-locus in the pistil. The S-alleles encode high basic proteins – glycoproteins with ribonuclease activity (McClure et al. 1989), thus they are often called S-RNases. These RNases specifically interact with similar S-locus in the male partner (Golz et al. 2001) – the pollen – and the recognition of relative or unrelated S-alleles in pollen direct the pollination and the fertilization. The pollination and the pollen-tube growth are inhibited, when the pollen and the pistil have the same S-allele. An S-alleles

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understanding can be used in an orchards design, because only some varieties are good pollinators. A bad choice of pollinators can cause lower yields and production.

Another trend of apple breeding is obtaining of varieties with a resistance against the apple scab (*Venturia inaequalis* CKE.). The dominant *Vf* allele derived from *Malus floribunda* Sieb. clone 821 is mostly used in resistance breeding (Dayton et al. 1970). Tartarini et al. (1999) described the co-dominant PCR marker of *Vf* gene detection. Vejl et al. (2003) and Melounová et al. (2004) evaluated the Czech apple varieties collection by PCR method.

**MATERIAL AND METHODS**

**Plant material**

Twenty-one Czech varieties were used for A-alleles, S-alleles and *Vf* gene analyses (Table 1). The following varieties were chosen as a reference: Skin colour (Cheng et al. 1996): Empire (*A*1*A*1), Esopus Spitzenburg (*A*1*a*1), Gala (*A*1*a*2), and Golden Delicious (*a*1*a*2); For S-alleles controlling incompatibility (Janssens et al. 1995): Ontario (*S*1*S*8), Golden Delicious (*S*2*S*3), Gala (*S*2*S*5), Idared (*S*3*S*7) and Jonathan (*S*7*S*9); For resistance against apple scab (Vejl et al. 2003): Varieties Denár (*vfvf*), Doris (*vf vf*), Resista (*Vfvf*) and Topaz (*Vfef*).

All of the analysed varieties were obtained from the gene source collection at the Research and Breeding Institute of Pomology in Holovousy.

**DNA isolation**

DNA isolation was realized from 100 mg of apple tissue by means of a DNA isolation kit (Qiagen, Germany). The quality and quantity of DNA was confirmed spectrophotometrically and electrophoretically.

**PCR amplification of A-alleles controlling skin colour**

The primers according to Cheng et al. (1996) (F-5’ GACAGGCTACGGTCCACTGCT 3’, R 5’ ACGTAAGTCAAGATTCAGATC 3’) were used

<table>
<thead>
<tr>
<th>Variety</th>
<th>Breeder</th>
<th>Year of registration in the CR</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneta</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>1998</td>
</tr>
<tr>
<td>Angold</td>
<td>Research and Breeding Institute of Pomology, Holovousy, CR</td>
<td>1995</td>
</tr>
<tr>
<td>Biogolden</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>2001</td>
</tr>
<tr>
<td>Goldstar</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>1998</td>
</tr>
<tr>
<td>James Grieve Red</td>
<td>Research and Breeding Institute of Pomology, Holovousy, CR</td>
<td>1970</td>
</tr>
<tr>
<td>Jantar</td>
<td>Jaroslav Lepeška, CR</td>
<td>1993</td>
</tr>
<tr>
<td>Jonalord</td>
<td>Otto Louda, CR</td>
<td>1993</td>
</tr>
<tr>
<td>Julia</td>
<td>Research and Breeding Institute of Pomology, Holovousy, CR</td>
<td>1994</td>
</tr>
<tr>
<td>Karmina</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>1995</td>
</tr>
<tr>
<td>Klára</td>
<td>Research and Breeding Institute of Pomology, Holovousy, CR</td>
<td>1994</td>
</tr>
<tr>
<td>Melodie</td>
<td>Otto Louda, CR</td>
<td>1991</td>
</tr>
<tr>
<td>Nabella</td>
<td>Research and Breeding Institute of Pomology, Holovousy, CR</td>
<td>1994</td>
</tr>
<tr>
<td>Nela</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>2001</td>
</tr>
<tr>
<td>Otava</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>1997</td>
</tr>
<tr>
<td>Rajka</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>1999</td>
</tr>
<tr>
<td>Rosana</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>1994</td>
</tr>
<tr>
<td>Šampion</td>
<td>Otto Louda, CR</td>
<td>1977</td>
</tr>
<tr>
<td>Selena</td>
<td>Research and Breeding Institute of Pomology, Holovousy, CR</td>
<td>1994</td>
</tr>
<tr>
<td>Sparjon</td>
<td>Jiří Červený, CR</td>
<td>2001</td>
</tr>
<tr>
<td>Vanda</td>
<td>Institute of Experimental Botany AS CR, Střížovice, CR</td>
<td>1994</td>
</tr>
<tr>
<td>Zuzana</td>
<td>Research and Breeding Institute of Pomology, Holovousy, CR</td>
<td>1997</td>
</tr>
</tbody>
</table>
The composition of the 25 µl reaction was: 50 ng of genomic DNA, 0.32µM of each primers, 1.5mM of MgCl$_2$, 0.3mM of dNTP and 1 unit of TAQ polymerase (Fermentas, Lithuania). The amplification was performed in the T-Gradient thermocycler (Biometra, Germany). The programme of the amplification was 40 × (94°C – 30 s, 60°C – 60 s, 72°C – 60 s) and 1 × (72°C – 480 s). The co-dominant PCR markers were electrophoresed through a 2% agarose gel and visualised by ethidium bromide (Sambrook et al. 1989).

### S-alleles PCR amplification

The PCR was performed according to Janssens et al. (1995) and Broothaerts (2003). Six S-alleles and one monomorphic fragment D were detected. The detection of S5, S7, S9 and D fragment was realized according to Janssens et al. (1995), and detection of S1, S2 and S3 according to Broothaerts (2003). The PCR composition was the same for all reactions; differences were only in the annealing temperature and the sequence of S-allele specific primers (Table 2). PCR amplification was performed with 25 µl reaction volume containing 100 ng of genomic DNA, 0.2µM of each primer, 1.5mM of MgCl$_2$, 0.2mM of dNTP and 1 unit of Taq polymerase (Fermentas, Lithuania). The annealing temperatures of the amplification profiles were optimised by means of thermocycler T-Gradient (Biometra, SRN). Table 2 shows the results of the optimisation. The programme of the amplification was: 1 × (94°C – 180 s), 30 × (94°C – 60 s, annealing temperature from Table 2 – 60 s, 72°C – 60 s), 1 × (72°C – 480 s).

Amplified PCR fragments were stained by ethidium bromide and electrophoresed through a 1.5% agarose gel.

### Vf gene PCR amplification

Primer pair for co-dominant Vf gene marker described by Tartarini et al. (1999) was used for a resistance evaluation. The PCR protocol according to Vejl et al. (2003) and Melounová et al. (2004) was applied.

### RESULTS

#### A-alleles controlling apple skin colour

The description of apple skin colour according to Sus et al. (2000) and Blažek (2001) shows Table 3. Cheng et al. (1996) used an explicit classification of green, yellow and red colour. The polymorphism of red/yellow skin colour is typical for many apple varieties. Red varieties are defined according to Cheng et al. (1996) as primarily (> 50%) red and yellow varieties as primarily (80–90%) yellow. The explicit classification of assessed Czech varieties is presented also in Table 3. PCR markers of al-

---

**Table 2. Primers and annealing temperatures used for PCR S-alleles detection**

<table>
<thead>
<tr>
<th>S-allele</th>
<th>Sequence of primers</th>
<th>Annealing temperature</th>
<th>Amplified fragment size</th>
</tr>
</thead>
<tbody>
<tr>
<td>S1 F</td>
<td>5’ ATATTGTAAGGCACCGCCATACATCAT 3’</td>
<td>60°C</td>
<td>530 bp</td>
</tr>
<tr>
<td>S1 R</td>
<td>5’ GGTTCGTATGGGGAAGACGCAAA 3’</td>
<td>60°C</td>
<td>449 bp</td>
</tr>
<tr>
<td>S2 F</td>
<td>5’ GTTCAACGTGACTTAGCCG 3’</td>
<td>49°C</td>
<td>375 bp</td>
</tr>
<tr>
<td>S2 R</td>
<td>5’ GTTITGGTCCTACATGACG 3’</td>
<td>58°C</td>
<td>1700 bp</td>
</tr>
<tr>
<td>S3 F</td>
<td>5’ CAAACGATAAAATACTTAC 3’</td>
<td>55°C</td>
<td>440 bp</td>
</tr>
<tr>
<td>S3 R</td>
<td>5’ TATATGGAAATCCACATTG 3’</td>
<td>62°C</td>
<td>343 bp</td>
</tr>
<tr>
<td>S5 F</td>
<td>5’ ATGAATTCTGCAAGGTCAACCG 3’</td>
<td>59°C</td>
<td>236 bp</td>
</tr>
<tr>
<td>S5 R</td>
<td>5’ ATGAATTCTGCAAGGTCAACCG 3’</td>
<td>59°C</td>
<td>236 bp</td>
</tr>
<tr>
<td>S7 F</td>
<td>5’ GTGGGATTCAATATCATC 3’</td>
<td>55°C</td>
<td>440 bp</td>
</tr>
<tr>
<td>S7 R</td>
<td>5’ GTGGGATTCAATATCATC 3’</td>
<td>62°C</td>
<td>343 bp</td>
</tr>
<tr>
<td>S9 F</td>
<td>5’ CAGCCGCTCGACTGACACCTT 3’</td>
<td>59°C</td>
<td>236 bp</td>
</tr>
<tr>
<td>S9 R</td>
<td>5’ CAGCCGCTCGACTGACACCTT 3’</td>
<td>59°C</td>
<td>236 bp</td>
</tr>
<tr>
<td>D F</td>
<td>5’ ATGCCCTCAGGCAGCCCTTTACC 3’</td>
<td>59°C</td>
<td>236 bp</td>
</tr>
<tr>
<td>D R</td>
<td>5’ ATGCCCTCAGGCAGCCCTTTACC 3’</td>
<td>59°C</td>
<td>236 bp</td>
</tr>
</tbody>
</table>
leles $A^1$ (1160 bp), $a^1$ (1230 bp) and $a^2$ (1320 bp) were found in the analysed Czech varieties. The electrophoreogram of separated PCR markers is shown in Figure 1.

**S-alleles controlling incompatibility.** Nowadays, 25 types of S-alleles were described in worldwide varieties. S1–S11 are more common alleles, the rest of S-alleles occurred only in single cultivars (Broothaerts 2003). The S-allele constitution was described in a majority of important varieties (Janssens et al. 1995, Van Nerum et al. 2001, Broothaerts 2003) and the presence of S2, S3, S5, S7 and S9 alleles was the most common. The described S-alleles analysis was chosen for this reason and this fact was confirmed in our experiments. The exemplary electrophoreograms of S2, S3 and S7 allele detection are shown in Figures 2–4. A PCR product of monomorphic D fragment (the conservative part of S-alleles) was obtained in all of the analysed varieties. The S-allele constitution is described in Table 4. A PCR product of monomorphic D fragment (the conservative part of S-alleles) was obtained in all of the analysed varieties. The S-allele constitution is described in Table 4.

**Vf gene detection.** Heterozygous genotype was detected in all of the resistant varieties. Susceptible varieties or varieties with a partial polygenic tolerance against the apple scab possess homozygous constitution of Vf gene. The electrophoreogram of a co-dominant PCR marker is presented in Figure 5. Detected allelic constitutions of all varieties are shown in Table 5.

**DISCUSSION**

**A-alleles controlling apple skin colour**

The A-alleles constitution was evaluated in 21 new Czech apple varieties. Blažek (2001) and Sus et al. (2000) presented the full characterization of apple skin colour. Nongenetic variability of the red respectively yellow colour intensity is very often. The apple skin colour was evaluated according to Cheng et al. (1996) for this reason. Only

### Table 3. Apple skin colour characteristic and detected constitutions of A-alleles

<table>
<thead>
<tr>
<th>Variety</th>
<th>Skin colour</th>
<th>Classification of skin colour according to Cheng et al. (1996)</th>
<th>Detected genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneta</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^1a^2$</td>
</tr>
<tr>
<td>Angold</td>
<td>yellowish-green with little red blush</td>
<td>yellow</td>
<td>$a^2a^2$</td>
</tr>
<tr>
<td>Biogolden</td>
<td>yellow without covering colour</td>
<td>yellow</td>
<td>$a^1a^2$</td>
</tr>
<tr>
<td>Goldstar</td>
<td>yellow without covering colour</td>
<td>yellow</td>
<td>$a^1a^1$</td>
</tr>
<tr>
<td>James Grieve Red</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^1A^1$</td>
</tr>
<tr>
<td>Jantar</td>
<td>mainly scarlet red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
<tr>
<td>Jonalord</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^1A^1$</td>
</tr>
<tr>
<td>Julia</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^1A^1$</td>
</tr>
<tr>
<td>Karmina</td>
<td>scarlet red colour on the whole fruit</td>
<td>red</td>
<td>$A^1A^1$</td>
</tr>
<tr>
<td>Klára</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^2a^2$</td>
</tr>
<tr>
<td>Melodie</td>
<td>mainly purple red covering colour</td>
<td>red</td>
<td>$A^1A^1$</td>
</tr>
<tr>
<td>Nabella</td>
<td>mainly carmine red covering colour</td>
<td>red</td>
<td>$A^1A^1$</td>
</tr>
<tr>
<td>Nela</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
<tr>
<td>Otava</td>
<td>yellow with little orange blush</td>
<td>yellow</td>
<td>$a^1a^2$</td>
</tr>
<tr>
<td>Rajka</td>
<td>mainly fresh red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
<tr>
<td>Rosana</td>
<td>mainly carmine red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
<tr>
<td>Šampion</td>
<td>striped red colour</td>
<td>red</td>
<td>$A^1a^2$</td>
</tr>
<tr>
<td>Selena</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
<tr>
<td>Sparjon</td>
<td>mainly dark red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
<tr>
<td>Vanda</td>
<td>mainly fresh red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
<tr>
<td>Zuzana</td>
<td>mainly orange red covering colour</td>
<td>red</td>
<td>$A^1a^1$</td>
</tr>
</tbody>
</table>
four varieties from 21 evaluated apple varieties possessed yellow colour. Varieties Biogolden and Goldstar lacked the sharp covering colour or the blush. Varieties Angold and Otava had small red or orange blush. The absence of dominant $A^1$ allele was characteristic for all of yellow varieties. Cheng et
al. (1996) also described that varieties with a sharp yellow colour (Golden Delicious) do not have the dominant allele A$_1$. Variety Honeygold with a red blush has according to Cheng et al. (1996) mainly a yellow fruit skin and an absence of A$_1$ allele. Similar results were obtained in varieties Angold (little red blush, genotype a$_2^2$a$_2^2$) and Otava (little orange blush, genotype a$_1^1$a$_2^2$). Lancaster (1992)

Table 4. Incompatibility and detected constitutions of S-alleles

<table>
<thead>
<tr>
<th>Variety</th>
<th>Detected S-allele constitution</th>
<th>Parental combination</th>
<th>Parental S-allele constitution</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneta</td>
<td>S7S?</td>
<td>Šampion × ÚEB 1200/1</td>
<td>S?S? × S?S?</td>
</tr>
<tr>
<td>Angold</td>
<td>S2S?</td>
<td>HL A28/38 × Golden Delicious</td>
<td>S?S? × S2S3</td>
</tr>
<tr>
<td>Biogolden</td>
<td>S2S3</td>
<td>Golden Delicious × ÚEB 1200/1</td>
<td>S2S3 × S?S?</td>
</tr>
<tr>
<td>James Grieve Red</td>
<td>S5S?</td>
<td>mutation of James Grieve</td>
<td>S?S?</td>
</tr>
<tr>
<td>Jantar</td>
<td>S3S7</td>
<td>Golden Delicious × Jonathan</td>
<td>S2S3 × S7S9</td>
</tr>
<tr>
<td>Jonalord</td>
<td>S2S7</td>
<td>Jonathan × Lord Lambourne</td>
<td>S7S9 × S?S?</td>
</tr>
<tr>
<td>Julia</td>
<td>S9S?</td>
<td>Quinte × Discovery</td>
<td>S?S? × S10S25</td>
</tr>
<tr>
<td>Karmina</td>
<td>S2S5</td>
<td>Karmen × ÚEB 725/6</td>
<td>S?S? × S?S?</td>
</tr>
<tr>
<td>Klára</td>
<td>S2S3</td>
<td>Hvězdnatá × Hájkova reneta</td>
<td>S?S? × S?S?</td>
</tr>
<tr>
<td>Melodie</td>
<td>S3S7</td>
<td>Šampion × OR 38 T 16</td>
<td>S3S5 × S?S?</td>
</tr>
<tr>
<td>Nabella</td>
<td>S5S9</td>
<td>Nonnetit × Starking Delicious</td>
<td>S?S? × S9S19</td>
</tr>
<tr>
<td>Nela</td>
<td>S1S?</td>
<td>Prima × ÚEB 1200/1</td>
<td>S?S? × S?S?</td>
</tr>
<tr>
<td>Otava</td>
<td>S3S7</td>
<td>Šampion × Jolana</td>
<td>S3S5 × S?S?</td>
</tr>
<tr>
<td>Rajka</td>
<td>S5S7</td>
<td>Šampion × ÚEB 1200/1</td>
<td>S3S5 × S?S?</td>
</tr>
<tr>
<td>Selena</td>
<td>S2S?</td>
<td>Britemac × Prima</td>
<td>S?S? × S2S10</td>
</tr>
<tr>
<td>Sparjon</td>
<td>S7S9</td>
<td>Spartan × Jonared</td>
<td>S?S? × S7S9</td>
</tr>
<tr>
<td>Šampion</td>
<td>S3S5</td>
<td>Golden Delicious × Cox Orange Pipin</td>
<td>S2S3 × S5S9</td>
</tr>
<tr>
<td>Vanda</td>
<td>S5S7</td>
<td>Jolana × Lord Lambourne</td>
<td>S?S? × S?S?</td>
</tr>
</tbody>
</table>

S? – unknown S-allele
indicated that many yellow varieties display a distinctive blush of anthocynin pigmentation. Different genes in the anthocyanin biosynthetic pathway can activate a red blush formation.

The rest of the 17 varieties possessed a different intensity of red apple skin. The presence of a dominant $A^1$ allele was specific for all of the red coloured apple varieties. These results are fully congruous with the study of Cheng et al. (1996). Dominantly homozygous constitution $A^1A^1$ is characteristic in varieties with darkly red fruits (for example Karmina, Julia, Jonalord, Nela, Melodie, Nabella). Cheng et al. (1996) did not describe a relationship between dominantly homozygous $A^1A^1$ constitution and dark red colour of fruits. Opposite of this fact, Cheng et al. (1996) found that the constitution $A^2A^1$ is typical for dark red, respectively violet red apple varieties (for example Empire, Jerseymac, Jonathan, Macoun, Monroe).

Cheng et al. (1996) discovered the occurrence of a dominant $A^2$ allele controlling also the red skin colour. The donor of this $A^2$ allele is the red crabapple variety White Angel. White Angel’s pedigree contains Asian apple genotypes (Simon and Weeden 1991). A PCR marker of allele $A^2$ (1180 bp) was not found in any analysed Czech varieties. This result corresponds to the fact that Asia genotypes are not often used in Czech apple breeding.

### Table 5. Resistance against the apple scab and detected allelic constitutions of $Vf$ gene

<table>
<thead>
<tr>
<th>Variety</th>
<th>Presence of resistance</th>
<th>Donor of resistance</th>
<th>Detected genotype</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aneta</td>
<td>monogenic vertical resistance</td>
<td>ÚEB 1200/1</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Angold</td>
<td>partial polygenic tolerance</td>
<td>HL A28/38</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Biogolden</td>
<td>monogenic vertical resistance</td>
<td>ÚEB 1200/1</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Goldstar</td>
<td>monogenic vertical resistance</td>
<td>Vanda</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>James Grieve Red</td>
<td>susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Jantar</td>
<td>susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Jonalord</td>
<td>susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Julia</td>
<td>susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Karmina</td>
<td>monogenic vertical resistance</td>
<td>ÚEB 725/6</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Klára</td>
<td>susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Melodie</td>
<td>monogenic vertical resistance</td>
<td>OR 38 T 16</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Nabella</td>
<td>less susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Nela</td>
<td>monogenic vertical resistance</td>
<td>Prima and ÚEB 1200/1</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Otava</td>
<td>monogenic vertical resistance</td>
<td>Jolana</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Rajka</td>
<td>monogenic vertical resistance</td>
<td>ÚEB 1200/1</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Rosana</td>
<td>monogenic vertical resistance</td>
<td>Jolana</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Šampion</td>
<td>susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Selena</td>
<td>monogenic vertical resistance</td>
<td>Prima</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Sparjon</td>
<td>susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
<tr>
<td>Vanda</td>
<td>monogenic vertical resistance</td>
<td>Jolana</td>
<td>$Vf/f$</td>
</tr>
<tr>
<td>Zuzana</td>
<td>less susceptible</td>
<td>none</td>
<td>$v/f$</td>
</tr>
</tbody>
</table>

## S-alleles controlling incompatibility

Table 4 shows the origins of the studied varieties. Variety Jantar was derived from a cross of Golden Delicious (S253) × Jonathan (S759). The PCR analysis confirmed that Golden Delicious was the donor of S3 allele and S7 allele is originated from the Jonathan in the variety Jantar. The origins of both S-alleles were confirmed also in the variety Šampion (S355), where the donor of S3 allele was the Golden Delicious and the donor of S5 allele was the Cox Orange Pipin. Also it was found that the donor of S2 allele in varieties Angold and Biogolden was the Golden Delicious, the donor of S7 allele in
variety the Jonalord was the Jonathan, the donor of S3 allele in varieties Melodie and Otava was the Šampion, the donor of S9 allele in variety Nabella was the Starking Delicious, the donor of S5 allele in variety Rajka was the Šampion, the donor of S2 allele in variety Selena was the Prima and the donor of S9 allele in variety Sparjon was the Jonared. Blažek (2001) described Vanda and Rosana varieties as reciprocally very bad pollinators. This result was confirmed on a molecular level. Both varieties contain the S5 allele.

S1 allele was detected only in the variety Nela. Broothaerts (2003) also indicated the low frequency of S1 allele in a world apple variety collection. Varieties with unusual S-alleles can be good pollinators for varieties with frequent S-alleles.

**Vf gene detection**

Varieties Aneta, Biogolden, Goldstar, James Grieve Red, Jantar, Nela, Šampion and Selena have not been evaluated by means of Vf gene PCR marker yet. Varieties Aneta, Biogolden, Goldstar, Nela and Selena show the field resistance against the apple scab. Each of the resistant varieties contains botanic species *Malus floribunda* in their pedigree. Heterozygous constitution Vfvf was found in all of the resistant genotypes. Dominant homozygous constitution VfVf was not described in any Czech apple variety (Vejl et al. 1993, Melounová et al. 2004). Varieties James Grieve Red, Jantar, Šampion and Sparjon are susceptible and show recessive homozygous constitution of Vf gene. The result of PCR marking in varieties Angold, Jonalord, Julia, Karmína, Klára, Melodie, Nabella, Otava, Rajka, Rosana, Vanda and Zuzana corresponds with the evaluation according to Vejl et al. (1993). The Vm gene is another possibility of breeding apple varieties with monogenic vertical resistance against the apple scab (Cheng et al. 1998, Melounová et al. 2004). The important donor of Vm gene (OR 45 T 132) was not found in any of the assessed new Czech varieties. Czech resistance breeding based on Vf gene corresponds to the fact that the resistance of genotype Vfvf is not overcome by new races of *Venturia inaequalis* CKE.

**REFERENCES**


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ABSTRAKT

Alely řídící barvu slupky a inkompatibilitu u nových českých odrůd jabloní s odlišným stupněm odolnosti vůči Venturia inaequalis CKE.

Na molekulárně genetické úrovni byla hodnocena kolekce 21 vybraných českých odrůd. U čtyř odrůd byla detekována genotypová sestava a_1a_1, a_1a_2 a a_2a_2 korespondující se žlutým zbarvením plodů, u sedmnácti odrůd byla detekována dominantní červená barva. Dominantně homozygotní sestava A_1A_1 byla přítomna u sedmi odrůd, heterozygotní sestava A_1a_1 nebo A_2a_2 byla nalezena u zbytku testovaných odrůd. S-alely kontrolují inkompatibilní systém pestíku. Jejich detekce je možná pomocí PCR na základě specifických primerů. Šest S-alel (S1, S2, S3, S5, S7 a S9) bylo studováno u stejné kolekce odrůd jabloní. Tento příspěvek přináší nové poznatky v charakterizaci S-alel, neboť české odrůdy nebyly dosud na molekulární úrovni studovány. Obě S-alely byly popsány u dvanácti odrůd, u devíti odrůd byla nalezena pouze jedna S-alela. Současné v téže kolekci odrůd byla sledována přítomnost genu Vf. Deset odrůd vykazujících polní odolnost vůči strupovitosti mělo heterozygotní sestavu Vf genu. Všech jedenáct senzitivních odrůd mělo homozygotní sestavu vvf.

Klíčová slova: Malus; PCR; Vf genu; inkompatibilita; S-alely; barva slupky; A-alely

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