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SELF-INCOMPATIBILITY IN APRICOT (*Prunus armeniaca)*; NEW ACHIEVEMENTS AND MOLECULAR ASPECTS OF S-LOCUS ALLELE SEGREGATION

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Preface

Since 2002, the research focusing on fruit trees in Rosaceae family, has provided a lot of information about S-allele determinants, for both pollen and pistil part, and recently discovered *SFB* (S haplotype-specific F-Box) in Prunoide and *SFBB* (S haplotype-specific F-Box Brothers) in Maloideae is considerable. The investigations into RNase-mediated GSI are a prime example of the successful integration of basic research and agriculture. The research into two species of Solanaceae; tobacco and petunia among the first plant model systems, in the 1980s laid the foundation for a molecular understanding of the GSI system. Fortunately, the GSI (Gametophytic Self-Incompatibility) in Rosaceae was also found to be S-RNase mediated, thereby taking the lead in the research in to this agriculturally important botanical family. Moreover, it turned out that there was also a direct contribution of the studies in Rosaceae to basic research. Another aspect is the fact that the S-locus of Rosaceae is much smaller than that of Solanaceae. Of course, identification of the S-RNase-mediated GSI system in Scrophulariaceae further increases the research possibilities. This was skillfully demonstrated in the investigation of the pollen-determinant gene SLF(S-Locus F-box)/SFB, in which finding from all tree botanical families contributed to our current understanding of the system. In addition, purely agricultural needs, such as the identification of the S-haplotypes of cultivars, led to the cloning of numerous S-RNase alleles. More recently, SLF/SFB alleles have been cloned that in turn contribute to the exploration of these genes. Cloning genes of the GSI system and understanding their role also provides tools for manipulating the system for agricultural needs. Naturally, this kind of genetic manipulation, which until now has only performed in apple (Broothaerts et al., 2004), will have to face the enduring debate on genetically modified edible fruit. While the structure of S-locus and roles of the genes located in it are known, the relationships among this locus and other genes important for pollen tube growth or rejection are still unknown. To clear all aspects of alleles interaction it is important to find synergies among three research approaches; Genetics, biochemistry and physiology. This great effort should produce knowledge available for breeding.

Introduction

Apricot (*Prunus armeniaca* L.), native to China and Central Asia and widely distributed over the world, has been attracted for its high drought resistance and obvious commercial prospect. The world production of apricot is 2,500,000 tons (FAOSTAT[1], 2007), with Turkey (557572 MT), Iran (280.000 MT), Pakistan (240192 MT), Uzbekistan (230000 MT) and Italy (214573 MT) being the main producers.

Apricots belong to the *Rosaceae*, genus *Prunus* L., subgenus *Prunophora* Focke, and section *Armeniaca* (Lam.) Koch (Rehder 1967). There are six species: *P.brigantina* Vill. (Briancon apricot) native to French alps; *P.armeniaca* L., apricot native to Asia and Caucasus; *P.dasycarpa* Ehrh, purple apricot, native to the former USSR; *P.mandshurica*(Maxim) Koehne, Manchurian apricot, native to Manchuria and Korea; *P.mume* (Sieb. & Zucc., Japanese apricot, native to Japan and China; *P. sibirica* L., Siberian apricot, native to eastern Siberia, Manchuria and northern China. Each species is diploid (*2n=16, x=8*) and all that have been studied are interfertile (Janick et al., 1996), but it seems that breeding of common apricot as other *Prunus* members except peach which is self fertile, is under the control of its self-incompatibility system for a large number of varieties. This system prevents successful crossing between cultivars possessing the same alleles. Moreover, cultivars of the same S genotype will not cross-fertilize each other in the orchard.

The trait of self-incompatibility:

Self-Incompatibility (SI) in flowering plants has been known for over a century. It is one of the most important systems used by many flowering plants to prevent self-fertilization and thereby generate and maintain genetic diversity within a species.) Firstly, it was described as a fascinating phenomenon (Darwin 1876). One-third to one-half of all flowering plant species are self-incompatible (Bian X.-Y., 2001).

Gametophytic Self- Incompatibility (GSI) , has been described in more than 60 families including Rosaceae (Igic and Kohn, 2001). This trait is controlled in Rosaceae by a homomorphic, gametophytic, monofactorial, multi-allelic

[1] http://faostat.fao.org/site/339/default.aspx

incompatibility system and pollen rejection occurs in the style (Sanzol and Herrero, 2002). Fruit production in many tree fruit crops is dependent on cross-pollination between cultivars due to the existence of a self-incompatible mechanism. There is a strong interest in the self-fertile character in many fruit and nut tree crops. This is apparent in sweet cherry and almond (Broothaerts et al., 2004).

Knowledge of S-alleles has become with time critical to establish cross-incompatibility between cultivars. All molecular-based plant SI systems analyzed to date are regulated by a number of multiallelic, genetically linked genes that confer recognition specificity (Nasrallah, 2005). One of the most intensively studied systems is that of S-RNase mediated gametophytic SI (GSI). In GSI, the pollen tube is arrested in the style if its *S*-haplotype (S-haplotype denotes the S-locus variant) matches either of the S-haplotypes carried by the pistil (McCubbin et al., 2000).

Molecular research into this system was initiated in *Nicotiana alata*, solanaceae, in which pollen rejection by the style found to be governed by a haplotype-specific RNase, *S-RNase* (Anderson et al. 1986; McClure et al., 1989). Sassa et al. (1992) found the orthologue of S-RNase in Rosaceae. These two families include a number of cultivated species. The cultivated species of Rosaceae includes the rose, strawberry, pome fruits (apple, pear, quince), and stone fruits (almond, apricot, cherry, peach and plum).

Genetic segregation analysis has revealed that the GSI system is carried on a single locus (the S-locus). The S-RNase gene governs the stylar part as a deletion of it in Japanese pear affected the pistil function but not the pollen function (Sassa et al., 1997). The pollen part function which has been suggested to be regulated by additional gene(s) that is linked to the S-RNase gene, was first identified in *Antirrhinum* (Lai et al., 2002), and then in Japanese apricot, almond, and cherry (Entani et al., 2003; Ushijima et al. 2003). In all, the pollen specific S-gene, after called *SLF/SFB* (S haplotype-specific F-box protein), is just a few kilo base (kb) away from the *S-RNase*.

Although Solanaceae and *Prunus* species use a similar molecule as the pistil S determinant (*S-RNase*), clear differences have been reported for their pollen S. First, pollen S in *Prunus* ,*SFB,* shows much higher allelic diversity (66 to 82.5% amino acid identity; (Ikeda et al., 2004a,b) than pollen S *(SLF)* in Solanaceae (88.4 to 89.4% amino acid identity; (Sijacic et al., 2004). Second, diploid pollen from the *Prunus* tetraploid is frequently capable of normal self-incompatibility function (Hauck et al.,

2006), but heteroallelic pollen from Solanaceae always shows breakdown of SI (competitive interaction;de Nettancourt, 2001). Finally, in Solanaceae, SLF (S locus F-box) is considered to be essential for pollen viability because all the pollen-part mutations were duplications of pollen S and no deletion type was recovered even after large-scale screening of X-ray induced mutants (Golz et al., 2001). In contrast, deletion of *SFB* results in pollen-part self-compatibility mutation in *Prunus* (Sonneveld et al., 2005). These differences in pollen S may reflect a mechanistic diversity of GSI systems among species.

In Rosaceae subfamilies; Spiraeoideae, Rosoideae, Maloideae, and Amygdaloideae, the GSI mechanism has been studied at a molecular level by several researchers and S-RNases have been characterized extensively; however, the pollen S gene (*SFB*) has been identified in *Prunus,* a species of Amlygdaloideae which seems to be different from pollen S in Solanaceae (Hauck et al., 2006). Characterization of pollen S in Maloideae and comparison of it to its counterparts in *Prunus* and Solanaceae is likely to shed light on the mechanism and evolution of the S-RNase-based GSI system. Recently, Sassa et al. (2007), have analyzed the S locus of two species of Maloideae: apple (*Malus x domestica*) and Japanese pear (*Pyrus pyrifolia*). Analyzing of the apple S9 haplotype revealed two similar F-box genes. Homologous sequences were isolated from different haplotypes of apple and Japanese pear, and they were found to be polymorphic genes in S locus.

- **S-RNase and SFB genes; structure and expression**

Genomic analyses for S-locus were first conducted on Solanaceous species such as *Petunia inflata, Petunia hybrida, Lycopersicon peruvianum*, and *Nicotiana alata*. However, the S-locus of these species is located in the sub-centromeric region and surrounded by abundant repetitive sequences that have hampered chromosomal walking (Entani et al., 1999b; Wang et al., 2003). Based on identification of pollen-only (pollen part) and stigma-only (stigma part) self-compatible mutants from Onagraceae and Rosaceae, Lewis (1951) first speculated that S locus has three separate but closely linked parts: an S allele specificity part, a pistil activity part and a pollen activity part. It has been common accepted now that the S locus consists of the pollen S and stigma S genes that are very closely linked but discrete genes.

S-RNase mediated GSI represents one of the largest allelic families in nature. Accordingly, since Sassa et al. (1992) identified the first Rosaceae S-RNase in Japanese pear, many more S-RNase alleles, from most of the economically important fruits-producing Rosaceae species, have been molecularly identified.

The S-RNase gene contains five highly conserved regions (C1 to C5) and a hyper variable region (HVa and HVb in solanaceae and RHV in Rosaceae), located between C2 and C3 (Ioerger et al., 1991; Ishimizu et al., 1998b; Sassa et al., 1997; Sonneveld et al., 2003), (Fig. 1). C2 and C3 share high sequence similarity with the corresponding regions of RNase T2 and RNase Rh (Horiuchi et al., 1988). A single intron is located between C2 and C3, except for *prunus* S-RNase alleles, which carry an additional intron between the secretion signal peptide and C1 (Beppu et al., 2002; Yamane et al., 2000). All the introns vary in size, although the variations in the one located between C2 and C3 are greater. Thus, PCR primers homologous to regions that flank the introns usually generate PCR products with different sizes that are characteristic of each allele.

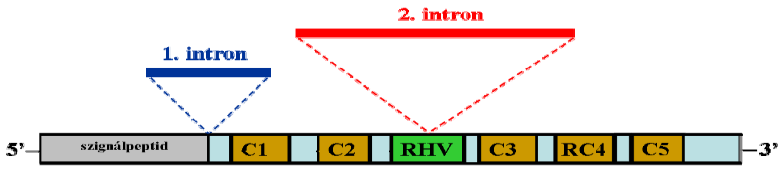


Figure 1– The proposed structure of S-RNase gene in Rosaceae fruit trees including five conserved regions (C1, C2, C3, C4 and C5) and Rosaceous hyper variable region (Sonneveld et al., 2003).

Based on the sequence of the different alleles, "allele- specific primers" generated to pair with variable sequenced of the allele, are also frequently applied (Zuccherelli et al., 2002; Romero et al., 2004; Sutherland et al., 2004; Halasz et al., 2005; Zhang et al., 2007; Goldway et al., 2009 and others).

S-RNases are expressed exclusively in the pistil, with the protein localized mostly in the upper segment of the style where inhibition of the self-pollen tubes advance occurs. The function of the S-RNases in SI has been directly confirmed by gain- and loss-of function experiments (Olden et al., 1968). These results clearly suggest that

the hyper variable regions play a key role in determining the S-haplotype specificity, despite the fact that the involvement of other regions cannot be ruled out. In the rosaceous S-RNase, the HVa region alone may form the interacting domain corresponding to the HVa and HVb regions of the Solanaceous S-RNases. S-haplotype-specific pollen rejection requires high levels of S-RNase expression. The concentration of S-RNase in the extracellular matrix is estimated at *10–50 mg/ml*, and only the transformants with an equivalent amount of S-RNase expression are able to acquire new S-haplotype specificities. The ribonuclease activity of S-RNases is essential for pollen rejection (Huang et al., 1994*)*. Furthermore, a radioactive tracer experiment showed that pollen RNA is degraded specifically after incompatible pollination (McClure et al., 1990). Thus, S-RNases function as highly specific cytotoxins that inhibit the growth of incompatible pollen. Although *S-RNase* is the sole female factor determining the S-haplotype specificity of the pistil, a requirement of other stylar factors for the full function of *S-RNase* has been suggested (Cruz-Garcia et al., 2003). One such factor is HT-B, firstly reported by McClure et al., (1999) in *Nicotiana,* a small asparagine-rich protein, pistil non S-factor. These results suggest that the HT-B protein is implicated in the SI response, although its exact function remains unclear (O'Brien et al., 2002).

The first clue for the male determinant was obtained from sequence analysis of the S-locus region of *Antirrhinum hispanicum*, a member of the Scrophulariaceae. The region of the S2-haplotype contained a novel F-box protein gene, *AhSLF-S2* (*A. hispanicum* S-locus F-box of S2-haplotype), which is specifically expressed in anther and pollen grains of S2-haplotype (Lai et al., 2002). Genomic analysis of the S-locus of *Prunus mume*, a member of the Rosaceae, reveals that the 60kb genomic region around the S-RNase gene contains as many as four F-box genes (Entani, et al., 2003). Among them, only one F-box gene, termed *PmSLF,* fulfills the conditions of a pollen S-determinant gene: (a) it is located within the highly divergent genomic region of the S-locus, (b) it exhibits S haplotype-specific diversity (78% to 81% amino acid identity), and (c) it is specifically expressed in pollen. Around the same time, polymorphic F-box genes were also found in the S-locus region of *Prunus dulcis, Prunus avium*, and *Prunus cerasus*, and were independently named *SFB* (S-haplotype-specific F-box; Ushijima K. et al., 2003; Yamane H. et al., 2003b). *SLF/SFB* from *Prunus* species fulfilled all conditions required of the pollen S determinant. Aligning deduced amino acid sequences of *SLF/SFB*s of these *Prunus*

species revealed the presence of two hypervariable regions, HVa and HVb, at the C terminus (Kao et al., 2004; Ushijima K. et al., 2004). Two self-compatible haplotypes of *P. avium* and *P. mume* encoded partial loss-of-function mutations in *SLF/SFB*, which lack both HVa and HVb regions (Ushijima et al., 2004). This fact provides additional evidence that the *SLF/SFB* is the pollen S-determinant (Fig.2).

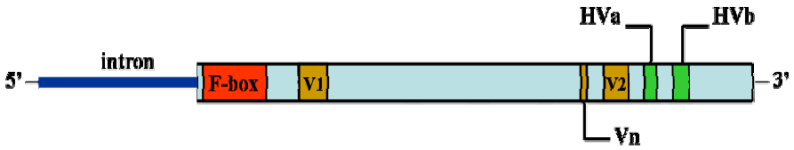


Figure 2- Schematic structure of SFB gene indicating F-box motif and Hypervariable regions (Ikeda et al.,2004a and Nunes et al., 2006).

- **Alleles Interaction**

In spite of the fact that both female and male determinants have been identified, the molecular mechanisms regulating how these molecules interact and specifically inhibit self-pollen growth are not completely explored. The fact that RNase activity is required for the function of S-RNases, and that S-RNases are taken up by both self- and nonself-pollen tubes, suggests that S-RNases function inside pollen tubes as specific cytotoxins degrading the RNA of self-pollen (Fig.3). On the other hand, *SLF/SFB* contains a motif, called the F-box, which is best known for mediating interactions with other proteins that make up an enzyme complex referred to as the E3 ubiquitin ligase complex (Gange et al., 2002). E3 ubiquitin ligases act in conjunction with the E2 enzymes to ubiquitinate
target proteins, which in many cases are degraded by the 26S proteasome. Although such interaction and degradation have not been reported for *Prunus SLF/SFB*s, if this is the case then *SLF/SFB*s should interact with all S-RNases but ubiquitinate only nonself-S-RNases. To explain the molecular mechanisms for this specificity, some hypothetical models compatible with the "inhibitor models" have been presented (Entani et al., 2003; Qiao et al., 2004; Sijacic et al., 2004; Ushijima et al., 2004).

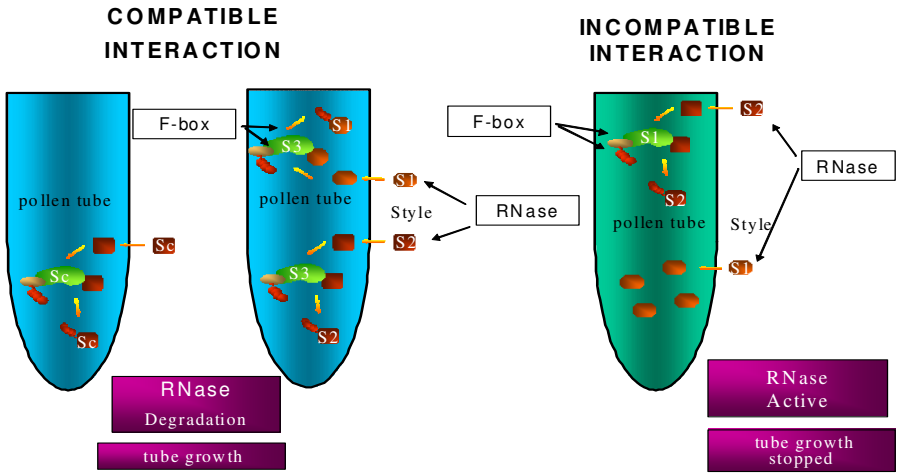


Figure 3– Proposing the *S1S2* genotype of pistil, in the incompatible interaction (right), both *S-RNases* are entered into the pollen tube with *S1* genotype, but the non-self one is recognized by *SFB* and will ubiquitinated for later degradation via 26S proteasome pathway and the self S-RNase enzyme is free and active which can degrade the mRNA necessary for protein synthesis. On the other side (centre and left) in the compatible interaction, the *SFB* can not recognize the non-self RNases and therefore forms a target complex with the non-self *RNases* for the next degradation , then pollen tube can grow toward ovary in the absence of RNase activity (Sansavini et al., 2009).

- **Factors other than S-RNase and SFB genes modifying the compatibility behavior**

Besides the above mentioned types of recognition/rejection mechanisms, there are another hypothesis considering contribution of other components. In addition to the S-RNase, HT-B and a 120KDa glycoprotein, which are style-expressed proteins, are needed for expression of self-incompatibility. In this model it seems that S-RNase is sequestered in a vacular compartment in compatible pollen tubes.

Down regulation of either of these proteins makes self-incompatible plants incapable of rejecting pollen. Investigation by Goldraij et al. (2006), to see the role of these proteins, they found S-RNase, HT-B and 120KDa protein were all introduced into both compatible and incompatible pollen tubes. In compatible pollen tubes, and in the early stages of incompatible pollinations, the S-RNase appeared to be sequestered in a vacuolar compartment that was bounded by the 120KDa glycoprotein. HT-B appears to be degraded in compatible pollen tubes. While this compartment appears to break down in incompatible pollen tubes late in pollination. In antisense HT-B plants, which

are completely self-compatible, S-RNase remains sequestered. Another protein non S-factor putatively involved in SI and characterized in pear is transglutaminase which plays a key-role in pollen tube growth (Di Sandro et al., 2008). How this gene is modulated inside the SI mechanism is still unknown.

- **Breakdown of Self-incompatibility in Rosaceous fruit trees**

From breeding point of view it is a valuable event that breakdown can happen in self-incompatibility behavior. Polyploidy and mutation break down SI. In contrast to its evolutionary advantage, SI is an unfavorable character in crops grown for fruits or seeds.

As mentioned in previous part, in most self-compatible fruit tree genotypes, self-compatibility is attributable to natural or artificially induced loss-of-function mutations in either the pistil or the pollen gene. In Japanese pear (*Pyrus serotina* ; Sassa et al., 1992) and almond (*Prunus dulcis*; Boskovic et al., 1997), self-compatibility was conferred by the failure of RNase protein expression or the loss of RNase activity of the mature proteins. Both transgenic approaches and analysis of spontaneous mutants demonstrate that eliminating the catalytic ribonuclease activity of the S-RNase, eliminate the ability to reject pollen (Sassa et al., 1997; Broothaerts et al., 2004). Self-compatibility may also be the consequence of a mutation within the F-box gene. Pollen-part mutant haplotypes of SC cultivars of sweet cherry (*Prunus avium*; Ushijima et al., 2004; Sonneveld et al., 2005) were artificially produced by X-ray irradiation. A deletion of the complete SFB3 gene or a frame-shift mutation in SFB4 resulted in the loss of pollen function of these two alleles. A naturally occurring SC haplotype (Sf) was characterized in Japanese apricot (*Prunus mume*) to be also a pollen-part mutant haplotype (Ushijima et al., 2004). A 6.8-kbp insertion in the middle of the SFBf coding region generated a stop codon and it is suspected that the HVa and HVb hypervariable regions, essential for allele-specific recognition, are missing (Ikeda et al., 2004). In addition, a pollen-part mutation is presumed to be present in the *Se* haplotype carried by all SC cultivars of Japanese plum (*Prunus salicina*; Beppu et al., 2005).

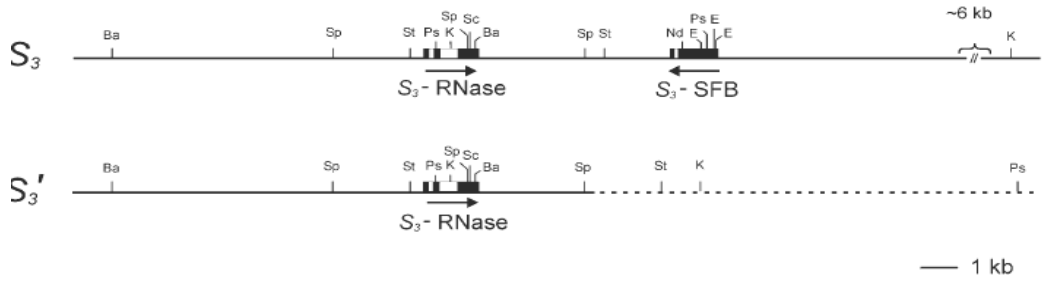


Figure 4-Preliminary restriction maps of *S3*- and *S3*'-haplotypes of cherry of 'Napoleon' *(S3S4*) and 'JI 2434'*(S3'S4*), A deletion of the complete SFB3 gene (Sonneveld T. et al. 2005).

The *S30* self-compatible mutant in *P. cerasus* also shows alterations in the SFB gene (Tsukamoto et al., 2006). In this allele, a 1 bp Guanine to Thymine substitution at position +733 produces a UAA stop codon that truncates the SFB protein and eliminates the HVa and HVb regions. Two additional *SFB* mutations have been reported in self-compatible peach, *Prunus persica*. SFB1 contains a 155 bp insertion that results in a truncated SFB protein, while SFB2 has a 5 bp insertion that produces a stop codon in the middle of the protein, truncating the protein upstream of the HVa and HVb regions (Tao et al., 2007).

Together, the identification of different pollen-part mutants in these species provides strong support for the identification of *SFB* as the pollen-component of GSI in the Rosaceae.

All self-compatible European apricot cultivars analyzed to date, carry the SC haplotype, long suspected and recently confirmed to be a pollen-part mutant haplotype, with a 358-bp insertion in the SFB gene (Vilanova et al., 2006, Halasz, 2007). Apricot S8, S9 and SC haplotypes were analyzed using a multilevel approach including fruit set evaluation, pollen tube growth analysis, RNase activity assays, polymerase chain reaction (PCR) analysis and DNA sequencing of the S-RNase and SFB alleles. *SFB8* was revealed to be the first known progenitor allele of a naturally occurring self-compatibility allele in *Prunus*, and consequently *SC=S8'*.

- **The structure of apricot S-locus**

To facilitate gene discovery in apricot, a bacterial artificial chromosome library (BAC-library) has constructed from the cultivar GOLDRICH, as an initial step towards the identification of the pollen S-gene. This work has accomplished by Romero et al., 2004, who analyzed the S-locus structure and identified the S-RNase

and SFB genes of apricot. For these analyses they used the cultivars GOLDRICH (*S1S2*) and HARCOT (*S1S4*). Their results showed that the S-locus genomic structure in apricot (*Prunus armeniaca)* is similar to those of other *Prunus* species reported to date.

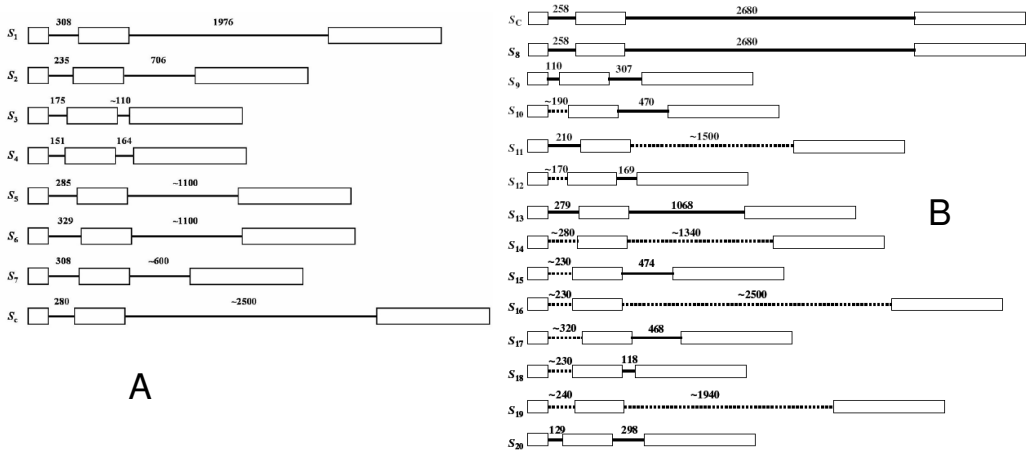


Figure 5- Schematic structure proposed for *S-RNase* in apricot demonstrating the polymorphism in intron size for recently identified alleles. The lines and boxes are demonstrating the introns and exons respectively. The conserved regions are sited in boxes (A:Vilanova et al., 2005, B: Halasz, 2007).

The apricot *S-RNases* have the typical features of *Prunus* T2-type RNases with five conserved domains (C1, C2, C3, RC4, and C5) and one hypervariable region (RHV). Motifs necessary for the RNase activity surrounding the histidine residues are present in the C2 and C3 domains. Apricot S-RNases like other known *Prunus* S-RNases contain two introns. The first intron is located within the junction between the signal peptide and the mature protein and the second one within the hypervariable region (RHV). The size of these introns vary in an S-haplotype manner. Romero et al., 2004, determined the length of these introns within *S1*, *S2* and *S4* alleles. The three studied S-RNase alleles show an amino acid identity with a mean of 77.5%. this high sequence polymorphism supports these genes as candidate for pistil determinants.

Apricot *SFB*s are also similar to the Prunus SFB alleles with one F-box domain, four (hyper)variable regions (V1, V2, HVa and HVb) and with an intron upstream of the

coding sequence. *SFB1, SFB2* and *SFB4* are also highly polymorphic with a mean of 79.4% amino acid identity.

Organ-specific expression studies provided additional evidence for *S-RNases* and *SFBs* being the pistil and pollen components, respectively, of self-incompatibility system in apricot, revealing that *S-RNases* are expressed in style tissues but not in pollen or leaves. In contrast, *SFB*s are exclusively expressed in pollen and not in leaves or styles.

- **Self-incompatibility model in apricot**

In almond a pistil part mutation resulted in an inactive *S-RNase* molecule, while in sweet cherry and Japanese apricot self-compatible pollen part mutants carry defective *SFB*s that were hypothesized to polyubiquitinate not only the non-self *S-RNases* but also the cognate *S-RNases* for subsequent degradation by 26-S proteasome pathway (Ushijima et al., 2004). In apricot a pollen-part mutation within the F-box region (a failure in pollen function) could be proposed in the self compatible cultivars. Recently, the pollen component gene of the self-compatibility haplotype, *SFBc*, was isolated and shown to carry a 358 bp insertion, which results in loss of function of pollen S. This was further supported by the identification of the original, non mutated form of apricot *SFBc*- allele (Halasz et al., 2007).

Sonneveld et al., 2005, proposed a model of *S-RNase* and *SFB* interaction that suggests a role for *SFB* proteins to prevent self *S-RNases* from being degraded and not recruit non self *S-RNases* for degradation as it was dictated by other models. By this model, self-incompatibility in apricot may be attributed to these procedures: the Sc-RNase and the mutated F-box protein are not able to form a stable complex, so the S-RNases will consequently be polyubiquitinated and degraded, which will result in fruit set.

- **Recent findings in apricot self-(in)compatibility**

The last five years supplied valuable information regarding the molecular bases of SC in apricot. Halász et al.,2007, extended the number of identified and characterized apricot S-alleles to a total of twenty. In the recently published paper by J.WU, 2009, eight new identified *S-RNases* are submitted to GeneBank and are denoted sequentially as *Par-S23, Par-S24, Par-S25, Par-S26, Par-S27, Par-S28, Par-S29, and*

Par-S30 (Table 1). Therefore the number of s-alleles in apricot is extended to a total of thirty. The new allelic variations are expected by examining the new and not yet studied apricot accessions from different origins.

Table 1- The newly discovered S-RNase and SFB alleles (J.WU, 2009).

New *S-RNase* allele	Accession number in Genbank	New *SFB* allele	Accession number in Genbank
Par-S_{23}	EU037262	*Par-SFB_{8}*	EU652883
Par-S_{24}	EU037263	*Par-SFB_{9}*	EU935588
Par-S_{25}	EU037264	*Par-SFB_{11}*	EU652884
Par-S_{26}	EU037265	*Par-SFB_{17}*	EU652885
Par-S_{27}	EU836683	*Par-SFB_{23}*	EU652886
Par-S_{28}	EU836684	*Par-SFB_{24}*	EU836685
Par-S_{29}	EF185300	*Par-SFB_{25}*	EU836686
Par-S_{30}	EF185301	*Par-SFB_{26}*	EU652887
		Par-SFB_{27}	EU836687

Recently the importance of *SFB* based S-genotyping has been considered comparing with the S-RNase based analyses. New approaches are employed to study the S-locus in apricot Using real-time fluorescence quantification RT-PCR technology, spatio-temporal expression patterns of S-RNase gene between SC and SI cultivars has been compared (Feng et al., 2006a). The usage of DNA chromatography (denaturing high-performance liquid chromatography – DHPLC) for identifying the S-genotypes of European apricots on the basis of their SFB alleles has reported by Raz and et al., 2009. Accordingly the specific primers for several S-alleles in apricot are designed (Table 2).

Table 2- The newly designed primers for apricot *S*-PCR (J.WU, 2009)

Primer name	Sequence (5' to 3')[†]	Note
S-8SRF	CTCCCTATTGTACTCCTAATGCCC	Specific primer for 3'-terminus of S_8-*S-RNase*
S-9SRF	AAGGTTGTACTTTTTTCTTTGGCC	Specific primer for 3'-terminus of S_9-*S-RNase*
S-11SRF	TAATGGGGAAAAAACCAATCTAAT	Specific primer for 3' -terminus of S_{11}-*S-RNase*
S-17SRF	GGTATGCTAGATGAAATTTTGAGG	Specific primer for 3' -terminus of S_{17}-*S-RNase*
S-26SRF	ATTGTGGTGCCATCCATGTGCTAT	Specific primer for 3'-terminus of S_{26}-*S-RNase*
Par-SFBF2	CCAAGCAAGTTCTTGANACAGG	Consensus primer for 3'-terminus of *SFB* alleles in *P. armeniaca*

[†] M = A/C; Y = C/T; R = A/G; K = G/T; W = A/T; S = C/G; H = A/C/T; and N = G/A/T/C.

Physical distance between *S-RNase* and *SFB* alleles in apricot has determined to indicate the tight genetic linkage between *S-RNase* and *SFB* which is one of the factors necessary for the S-RNase-based GSI system, in order to suppress recombination between the pollen S-determinant gene and the pistil S-determinant gene. To address the direction of transcription and the linkage relationship of the S-*RNase* and *SFB* genes, J.WU et al., 2009, carried out specific PCR amplifications of the S8, S9, S11, S17, and S26-haplotypes using specific primers for each S-RNase and consensus primers for SFB (Table 2). The results showed that each primer pair (i.e., a forward primer PsSFB-F2 for SFB in combination with a forward primer specific for each S-RNase) gave PCR amplifications of 900 – 2,000 bp in four S-haplotypes (S9, S11, S17, and S26). The amplification products were cloned and sequenced, and corresponded to the separate SFB and S-RNase sequences, implying that the two genes were physically linked, with opposite transcription orientations. The physical distance determined between the S-RNase and SFB genes for the S9, S11, S17 and S26-haplotypes were 620, 299, 1,061, and 387 bp, respectively.

The phylogenetic analysis of newly identified apricot and other *Prunus* S-RNase alleles confirms their trans-specific evolution within the *Prunoideae* subfamily.

- **The contribution of self-incompatibility in apricot breeding programs:**

According to the list of majore objectives in apricot breeding programs published by Bassi et al., 2006, the high percentage of self-incompatible genotypes which arose from breeding programs has considered as one of main objectives . Regarding to the practical aspects of the self-incompatibility, from either orchard management or variety improvement point of view, all activities are related to self/cross

compatibility. Floral biology studies are necessity to plan hybridization programs . Determining the mode of inheritance of productivity related traits improves the efficiency of breeding. For instance, male sterility may produce up to 25% of male-sterile seedlings from crosses between fertile heterozygous cultivars (Burgos 2004), Therefore appropriate parental selection is the solution. Also, determining the inheritance of self-(in)compatibility and the parents genotypes for this trait allows hybridizations to be planned which minimize or eliminate the production of self-incompatible seedlings. The correlation between stylar *RNases* and different S-alleles has been a great advance for determination of the genotype of a good number of cultivars. With this methodology, homozygous self-compatible cultivars can be easily identified, which will produce 100% self-compatible progeny regardless of the other parents genotype. If the necessity of evaluating the progenies generated within the breeding program, to discard the self-incompatible seedlings, is eliminated, the program is speeded up, which greatly reduces its cost. Self-incompatibility phenotype determination by controlled crosses and evaluation of fruit set or pollen tube growth as well as *RNase* analysis, to determine the genotype at the *S* locus, need mature trees with flowers, which, for fruit trees, means at least three years after seeds are obtained. Using PCR with *S*-allele-specific primers allows detection of the self-incompatible genotype in the first stages of plant development, and therefore allows rouging of undesirable seedlings straight after germination of the seeds. Specific primers to amplify selectively the allele (or alleles) that determine self-compatibility are molecular markers for this trait with 100% efficiency, since they are located within the *S* locus. In apricot, these primers have not yet been developed specifically for all alleles. However, some recent papers on this species, and methodologies developed in related *Prunus* species, indicate that they will soon be available. The number of publications in recent years indicates the interest in the different aspects of reproductive biology. This interest is, possibly, closely linked to the fact that this knowledge may avoid production failures and also allows the efficiency of the fruit breeding programs to be increased.

CHAPTER 1:

Case Study

1.1 Statement of Problem:

Although cross-pollination at an evolutionary scale is profitable for plants, it is undesirable trait for growers because pollen donor trees must be provided in the orchards of self-incompatible cultivars. In addition, self-incompatible cultivars carrying the same S-genotype could not fertilize each other which mean cross-incompatibility. Most fruit tree cultivars that belong to the Rosaceae are self-incompatible and depend on cross-pollination. The pollen donor and pollen recipient have to flower synchronously and must be genetically compatible.

Fruit trees belonging to the Rosaceae family are characterized by gametophytic self-incompatibility which is governed by ribonucleic enzymes (RNase) and SFB proteins. There are rather a high percentage of self-incompatible genotypes in apricot. Self-incompatibility of apricot varieties belonging to different eco-geographical groups has been screened by several researchers. According to Guerriero and Bartolini 1995, the majority of the European varieties are self-compatible, however, most of the North-African, Middle-Eastern, Central Asian and Irano-Caucasian varieties are self-incompatible, but the number of self-incompatible varieties is increasing. In the last years many self-incompatible cultivars were released from breeding programs using Asian and American genotypes to introduce frost tolerance and virus resistance. Therefore, knowledge on the S-genotypes is necessary for planning the orchards.

However, apricot breeding and production has been hampered by the trait of self-incompatibility. From horticultural point of view there is a strong interest in self-fruitful cultivars. The need of pollinators, with overlapping blooming times, as well as pollinating insects to transfer the pollen; make self-incompatible cultivars unsuitable to modern horticultural practices. Knowledge on the inheritance of this trait and methodologies to determine the genotypes of different cultivars as soon as possible,

have allowed the planning of hybridizations so that the number of self-incompatible seedlings is minimized in the progenies from controlled crosses. Thus, the S-genotype of cultivars is an important feature and is characterized molecularly by the *S-RNase* and *SFB* alleles which are distinctive for each S-haplotype.

1.2 Purpose of the Study:

Open field experiments to determine compatibility relations give doubtful results because environmental and weather conditions can greatly influence fruit set ratios, and mature trees are required for these analyses. DNA based analysis can support breeders' work by providing the possibility for the early selection of seedlings carrying the favorable trait. Besides knowledge on the S-genotypes of self-incompatible cultivars presents useful information for the optimal cultivar association, elucidation of the molecular mechanisms controlling the fertility trait of fruit trees is also a very important task of basic research. Although multi-approached studies seems to be more efficient to confirm the results in parent selection during hybridization programs but at large scale during screening seedlings for self-compatibility trait, it seems that the molecular marker based screening is very rapid and accurate.

This experiment designed at Department of Fruit and Woody Plant Sciences of Bologna University to cover the following objectives:

a) To evaluate the right situation about S-locus of the utilized genotypes.

b) To verify if the apricot S locus is similar to that of the *Prunus* species.

c) To examine an easy to apply, rapid and reliable tool for breeders that enables the early selection of seedlings for self-compatibility.

d) To determine and characterize as many as possible allelic variants of the *S*-locus by screening a group of Italian, European, North American and Iranian varieties and assign *S*-genotypes for economically important apricot cultivars.

e) To establish inter-fertility groups among apricot cultivars by comparing the results with the data published previously. This tool would be used as a genetic database by both growers and breeders.

f) To describe the self-compatibility trait segregation among the crosses and detect the original, functional version of the self-compatibility.

1.3 Description of Terms:

All known S-genotyping procedures, i.e. the classical fruit set analysis after open field test crosses; pollen tube growth monitoring with fluorescent microscopy; stylar ribonuclease electrophoresis (not applied in this work); as well as the most recent polymerase chain reaction based DNA-level analyses and DNA sequencing have been applied by different researchers worldwide. Here the procedure of all approaches is described with a mention on their advantages and disadvantages. Part of descriptions is adopted from Halasz et al., 2006.

1.3.1 Classical methods for *S*-genotyping:

1.3.1.1 The field pollination and fertility studies: Evaluating fruit set after controlled pollination has been the only way to assign S-genotypes to cultivars for so long. The technique itself is simple; however, it has a lot of shortcomings. Before pollination, pollen is collected from all tested cultivars by desiccating the anthers in a Petri dish at ambient temperature. In the orchard, shoots must be chosen that seem to have an adequate number of flowers at balloon stage, open flowers and late flower buds must be removed. Flowers are emasculated to prevent self-pollination. Controlled pollinations in the required combinations and directions are carried out by a glass rod, toothpick or merely by fingers when stigmata are receptive and completely covered by exudates (*Guerriero & Bartolini*, 1995). After about eight weeks, the resulting fruits are counted and the fruit set percentage is determined. To determine self-compatibility, two methods are used generally (*Nyujtó* et al., 1985; *Burgos* et al., 1993; *Nyéki*, 1996; *Nyéki & Szabó*, 1995). In artificial self-pollination studies, pollen transfer is carried out using pollen of the same cultivar. Autogamy which means bagging branches with closed flower buds and the determination of the percentage of fruit set obtained, the ability of a tree to yield fruits in the absence of pollinizer cultivars and pollinators can be assessed. This is thereby not so informative in cases, when we would like to confirm an *S*-genotype by field crosses. Geitonogamy may result more valuable data. It also means self-pollination, but by directly

allocating pollens to the stigmata, thereby failure in fruit set can only be attributed to incompatibility reactions. Bagging can be accomplished by water-proof parchment paper bags. Any open flowers at bagging must be removed, and buds are counted. Fruit set study is an extremely cheap and for results one must wait 1 or 2 months. Test crosses are generally required for the confirmation of molecular *S*-genotyping studies, and these are really useful for getting information on the function of the described systems, however, they may be hampered by several environmental factors. To eliminate these, crosses can be carried out in the laboratory, and results evaluated by means of fluorescence microscopy.

The greatest disadvantage of these procedures is that *S*-genotyping can only be achieved by a series of crosses, which is not easy to be realized and lasts for years due to the long juvenile phase of the fruit trees. At first, self-incompatibility must be verified by self-pollination. In a species with functional gametophytic self-incompatibility (GSI) system, cross-incompatibility can be found between several cultivars. Cultivars or seedlings belonging to the same inter-incompatibility group are characterized by the same *S*-genotypes.

Fruit set studies may not reflect a semi-compatible combination, as half of the pollen grains are able to fertilize the ovules, which can result in reasonable yields since many pollen grains may land on a stigma. This methodology was used in several fruit tree species to determine their *S*-genotypes. In sweet cherry, *Crane & Lawrence* (1929) tentatively assigned alleles to five incompatibility groups encompassing 19 cultivars. Later, *Matthews & Dow* (1969) published a list, in which six incompatibility alleles were variously assigned to some 140 cultivars in 10 inter-incompatibility groups. This technique was also successfully used for investigating the types of spontaneous and X-ray induced mutations that resulted in self-compatible seedlings (*Lewis & Crowe,* 1954). This methodogy have been applied in other fruits such as almond (*Tufts & Philp,* 1922). It must be mentioned that in case of apple or pear cultivars, this method may provide misleading results, since some of the cultivars tend to set fruits by parthenocarpy. In all cases analysis must be complemented by counting the seeds within fruits.

1.3.1.2 Following pollen tube growth with fluorescent microscopy: After the pistil is pollinated, the pollen grain germinates in a response to a sugary fluid secreted by the mature stigma (mainly sucrose). From each pollen grain, a pollen tube

grows out that attempts to travel to the ovary by creating a path through the female tissue. The vegetative (or tube) and generative nuclei of the pollen grain pass into its respective pollen tube. After the pollen grain adheres to the stigma of the carpel (female reproductive structure) a pollen tube grows and penetrates the ovule through a tiny pore called a micropyle. Before anthesis, mature pollen grains are dehydrated with water-contents ranging from 6 to 60% because it confers a tolerance to the environmental stresses on them and it may be a necessary prerequisite for pollen viability and subsequent germination (*Lin & Dickinson*, 1984). This process is reversible: reaching a suitable flower, so landing upon an appropriate stigma, pollen hydrates. Pollen hydration is tightly regulated and several molecules are known to be involved in stimulating it.

Once they are hydrated, pollen grains attain a distinct polarity and germinate to produce a pollen tube which grows by tip extension (*Franklin-Tong*, 2002). In many plant families where gametophytically controlled self-incompatibility exists, including *Rosaceae*, "wet" stigma type is found, which means that copious secretion fluid accumulates on the surface of stigma and forms a medium for the germination of the captured pollen (*Heslop-Harrison*, 1975).

Pollen tubes' travelling through the stylar tissues as all events of fertilization is a cooperative and highly organized process between the male and female partners. Growing through the style, the pollen tubes push away the mucilaginous cell walls, thus the cells lose their tension and collapse. That is the reason why the intruding pollen tubes do not induce the lateral expansion of styles. Pollen tube growth is heterotrophic at the expenses of the stylar reserves (*Herrore and Hormaza*, 1996), since the reserved substances in pollen grain are not sufficient for the growth of a pollen tube from stigma to ovule. A transmitting tissue specific glycoprotein was isolated and suggested to have nutritive role, since it was deglycosilated by in vitro-growing pollen tubes (Wu et al., 1995).

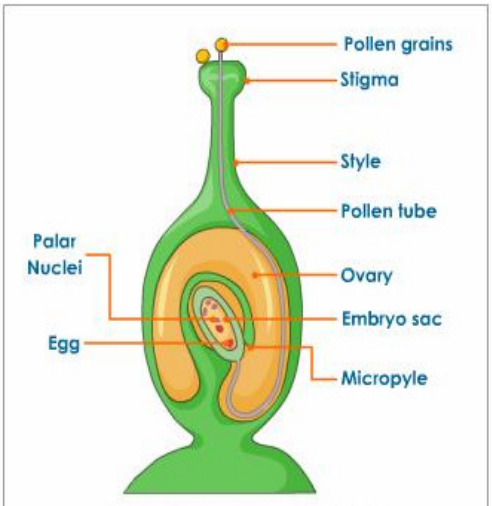


Figure 1.1- Fertilisation in a Flowering Plant

Pollen tube is an extremely specialized cell type, including a generative cell, which contains the two sperm cells, and the vegetative nucleus. It has a unique structure:

pollen tube is itself haploid and in fact it comprises a cell within a cell. The cytoplasm carrying the vegetative and sperm cells is located toward the growing tip of the front region. In this region there are also several other organelles, like mitochondria, endoplasmic reticulum and Golgi complexes. The cytoplasm is separated from the remainder of the pollen tube by callosic cross walls. These callose plugs are laid down at regular intervals as the tube grows and give the tubes a ladder-like appearance. During growth, the regions behind the callose plugs are vacuolated so the cytoplasm concentrated in the front portion of the tube, regardless of its length (Franklin-Tong, 1999). Pollen tubes' wall consists of two main layers of polysaccharide. The inner callosic wall contains predominantly (1,3)-β-glucan (Newbigin et al., 1993). It has been reported by de Nettancourt et al. (1973) that there is an increase in the callosic particles in the cytoplasm of pollen tubes after incompatible pollination and the same authors suggested that these callosic particles result from the breakdown of the inner pollen tube cell wall. According to Cresti & Went (1976) the increased number of callosic particles in response of an incompatible pollination might be due to a premature degeneration of the cytoplasm as a result of the inhibited growth of pollen tubes caused by the incompatibility reaction.

The fact that callose can be stained selectively with a water-soluble substance aniline blue or similar fluorochromes, was first reported by Currier (1957). The stained callose layer in either living or dead tissue will fluoresce intensively in ultraviolet light (Evans & Hoyne, 1982). Staining of pollen tubes with aniline blue reveals the presence of callose plugs through the style and provides an advantage over the vital stains previously used, by which the uniformly stained stylar cells and pollen tubes could have only been differentiated by some hardly visible structural properties. However, this outdated technique could also supply some data concerning S-genotypes of fruit trees.

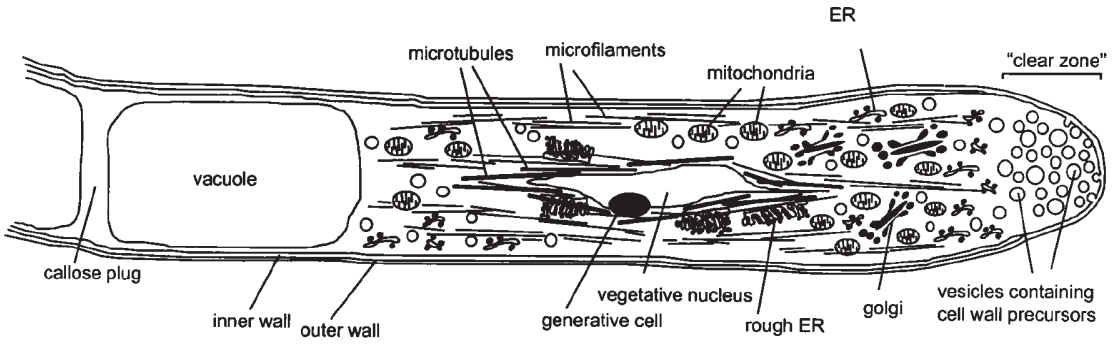


Figure 1.2- Generalized diagrammatic representation of a pollen tube (Franklin-Tong, 1999)

Kobel et al. (1938) had assigned incompatibility alleles to sweet cherry cultivars in Switzerland. They could also distinguish between compatible, semi-compatible and incompatible crosses and assign 11 S-alleles to some 20 apple cultivars (Kobel et al., 1939). Martin (1959) described the first appropriate staining technique of pollen tubes in style. His protocol comprised the following steps: styles are fixed in formalin : 80% alcohol : acetic acid for 24 hours or more. After rinsing in tap water, they are treated in an about 8 N sodium hydroxide solution for 8 to 24 hours to clear and soften the tissue and to permit adequate penetration of dye. Staining is accomplished in a 0.1% solution of aniline blue dye in 0.1 N K3PO4 for 4 hours.

For observations the stained styles are put on clean glass slides and are covered with cover slips. The slide must be directly illuminated by ultraviolet light using a conventional microscope in a darkened room.

Under these conditions all the sites along a pollen tube where a callose deposition is present, will fluoresce bright yellow to yellow-green, whereas the background tissue will fluoresce pale grey or blue. Varying amounts of callose occur frequently in the sieve tubes and within the epidermal hairs of the style but with some experience pollen tubes can be distinguished from them by their size, shape and distribution in the style. If these difficulties inhibit the clear observation of the tubes, it might be avoided by adequately modifying the above detailed procedure. Prepared slides can be sealed and stored for some months in a refrigerator at 4–5 °C. Success of the technique depends on the sufficient amount of callose. Callose distribution and

amount within the pollen tube wall was shown to be variable according to different species as well as several external factors. It sometimes appears through the entire tube; nearly fill its whole length. In other cases, callose is localized as closely spaced plugs. Rarely the amount of callose is too small to make visible the growing tube. It was found in pollen tubes of many self-incompatible species that the amount of callose might be higher after self- than cross-pollination (Linskens & Esser, 1957; Halász et al., 2004). In incompatible tubes, the pattern of growth is similar to that initially seen in a compatible pollination, but at some stage, growth becomes irregular, the pollen tube walls become thicker and the tips may burst, while the growth of compatible pollen tubes is unaffected, therefore they can successfully reach the ovary (Newbigin et al., 1993).

Almond was one of the first rosaceous species where this technique was expansively used (Socias i Company et al., 1976; Ben-Njima & Socias i Company, 1995). Dicenta & García (1993) also employed the classical method of Martin with some modifications. Prior to microscopic evaluation the pistils were washed, and treated in a 5% sodium sulphite (Na2SO3) in an autoclave at 1–1.2 kg cm-2 to soften the tissue and improve staining efficiency (Jefferies & Belcher, 1974). A further step was included: the pubescence was removed before pistils were placed on slides and squashing them. This method was also successfully employed by several research groups in case of many almond genotypes (Bosˇkovic´ et al., 1999; Ortega et al., 2002; Ortega & Dicenta, 2003; Socias I Company & Alonso, 2004; Ortega & Dicenta, 2004).

Apricot styles, just like those of almond, are also hairy; therefore pollen tubes could not be observed surely unless pubescence is completely removed (Burgos et al., 1993; Andrés & Durán, 1998). First data concerning apricot pollen tube growth was presented by Egea et al. (1991). Fixing pollen tube growth 72 h after pollination leaves enough time for tubes to reach the ovary in case of almond, but it proved to be insufficient for apricot: only 25% of compatible combinations had tubes reaching the ovary during 72 h (Viti et al., 1997; Audergon et al., 1999). Consequently, authors proposed to extend the time between pollination and fixation to 96 h in case of apricot. The method with slight modifications according to the properties of the adequate plant species was successfully used in case of apple, Japanese pear, European pear (Sanzol & Herrero, 2002).

Microscopic observation of pollen tubes is very difficult, even for experienced experts as callose deposits not exclusively occur in pollen tubes to be monitored. Although this technique is burdened with some shortcomings and does not enable direct S-genotyping, it remains to be a precious technique to verify molecular results obtained by stylar RNase detection or PCR analysis.

1.3.2. DNA-based analyses: PCR, sequencing

1.3.2.1 DNA extraction: Several DNA extraction protocols are used for the molecular analysis of plants. Usually, for S-PCR analysis two main types of DNA isolation methods are utilized. One of them is the protocol based on a detergent, called cetyltrimethylammonium bromide (CTAB), which forms an insoluble complex with nucleic acids (Doyle & Doyle, 1987). When CTAB is added to a plant cell extract, the nucleic acid–CTAB complex precipitates, leaving carbohydrate, protein and other contaminants in the supernatant. The precipitate is then collected by centrifugation and resuspended in 1 M NaCl, which causes the complex to break down. Thus the nucleic acids can be concentrated by ethanol precipitation and the RNA removed by ribonuclease treatment. A second method makes use of the fact that nucleic acid molecules, unlike most of the contaminants in a cell extract, have relatively strong negative charges. This means that nucleic acids bind to positively charged surfaces, for instance to the particles in an anion-exchange chromatography resin. The resin is placed in the column and the cell extract added onto it. Nucleic acids are retained in the column, whereas the neutral and positively charged contaminants pass straight through. After washing away the last contaminants, the nucleic acids are recovered by adding a high-salt solution, which destabilizes the electrostatic interactions between the nucleic acid molecules and the resin. This chromatographic method is the base of several kits, which are commercially available (e.g. Qiagen, Germany).

Using the CTAB method for DNA isolation has several advantages and disadvantages involving that it yields a DNA extract of high concentration and it is cheap, however, the obtained DNA solution will be more contaminated and the procedure is time-consuming. In contrast to it, using an extraction kit may provide a less contaminated DNA extract with an overall better quality, but its DNA content will be definitely lower compared to that of the extracts gained by the CTAB method, and these kits are rather expensive.

1.3.2.2 Polymerase chain Reaction (PCR): The method, which has become known as Polymerase Chain Reaction (PCR) first, was designed to obtain many copies of an arbitrary DNA sequence (the template) during a short period of time (Mullis & Faloona, 1987). It is necessary that the ends of the sequence be known in sufficient detail that two oligonucleotide primers can be synthetized, which will hybridize to them. The sequence to be synthesized can be present initially as a discrete molecule or it can be part of a larger molecule. In either case, the product of the reaction will be a discrete dsDNA molecule with termini corresponding to the 5' ends of the oligomers employed.

A source of DNA including the desired sequence is denatured and the oligonucleotide single-stranded primers hybridize to the edges of the target sequence, then a DNA polymerase enzyme will replicate the template DNA strand using the previously added four types of deoxyribonucleoside triphosphates. First report on the PCR amplification of S-alleles were described by Brace et al. (1993) using the sporophytic self-incompatible *Brassica oleracea*. Primers were constructed from known sequences and allele identification was carried out by digestion with several restriction enzymes. Taking advantage of the abundance of S-locus mRNA in stylar tissue, *Solanum carolinense* was the first gametophytic self-incompatible species from which S-alleles were amplified using reverse-transcriptase (RT-)PCR (Richman et al., 1995). Primer pairs were designed to the conserved regions of the solanaceous S-alleles.

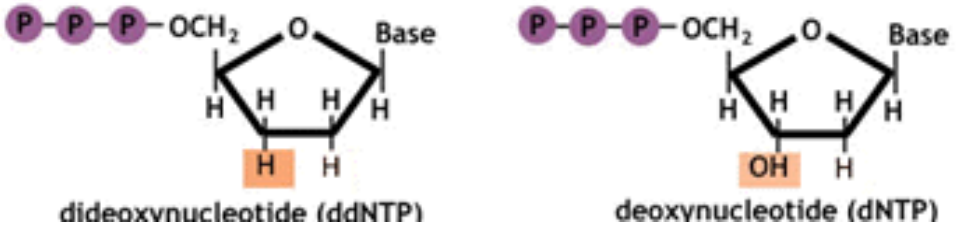
Research on the self-incompatibility of Rosaceae, which always lagged behind the studies on Solanaceae made up lost ground in 1995, since a very similar report was published in the same year involving cDNA cloning and PCR amplification of two alleles from apple (Broothaerts et al., 1995). Furthermore, shortly after this first report a new paper was published to identify three additional S-allele cDNAs of apple and develop a molecular technique for the diagnostic discrimination among the five different S-alleles of apple (Janssens et al., 1995). Many degenerate and allele-specific primers were designed for PCR analyses in several rosaceous fruit species (Tao et al., 1999; Tamura et al., 2000; Sonneveld et al., 2001; 2003; Sapir et al., 2004; Sutherland et al., 2004; Vilanova et al., 2005).

Pollen component of GSI system was identified in 2003 to be an F-box molecule, which takes part in the ubiquitin mediated proteolysis. First data on an F-box based S-genotyping method were provided by Yamane et al. (2003c) in Japanese apricot.

1.3.2.3 Sequence analyses: Since the development of methods of high-throughput production of gene and protein sequences during the 90s, the rate of addition of new sequences to the databases increases continuously. Such a collection of sequences, and comparing sequences with known functions with these new sequences is one way of understanding the right situation from which the new sequence comes. Thus, sequence analysis can be used to assign function to genes and proteins by the study of the similarities between the compared sequences. Nowadays there are many tools and techniques that provide the sequence comparisons (sequence alignment) and analyze the alignment product to understand the biology. The S-alleles have been sequenced since 1991 *Solanum* species.

originally two methods were invented around 1976 to determine DNA Sequence, but only one is widely used which invented by Fred Sanger[2]. This method uses DNA polymerase to synthesize a second DNA strand that is labeled. Recall that DNA polymerase always adds new bases to a primer.

Also uses chain terminator nucleotides: dideoxy nucleotides (ddNTPs), which lack the –OH group on the 3' carbon of the deoxyribose. When DNA polymerase inserts one of these ddNTPs into the growing DNA chain, the chain terminates, as nothing can be added to its 3' end.



The template DNA is usually single stranded DNA, which can be produced from plasmid cloning vectors that contain the origin of replication from a single stranded bacteriophage. Infecting bacteria containing this vector with a "helper phage" causes single stranded phage to be produced. The phage DNA contains the cloned insert. The primer is complementary to the region in the vector adjacent to the multiple cloning site. Sequencing is done by having 4 separate reactions, one for each DNA base. All 4 reactions contain the 4 normal dNTPs, but each reaction

[2]Source: http://www.bios.niu.edu/johns/humgen/DNA_Sequencing_and_Gene_Analysis.ppt

also contains one of the ddNTPs. In each reaction, DNA polymerase starts creating the second strand beginning at the primer. When DNA polymerase reaches a base for which some ddNTP is present, the chain will either terminate if a ddNTP is added, or continue if the corresponding dNTP is added. which one happens is random, based on ratio of dNTP to ddNTP in the tube.

However, all the second strands in, say, the A tube will end at some A base: you get a collection of DNAs that end at each of the A's in the region being sequenced.

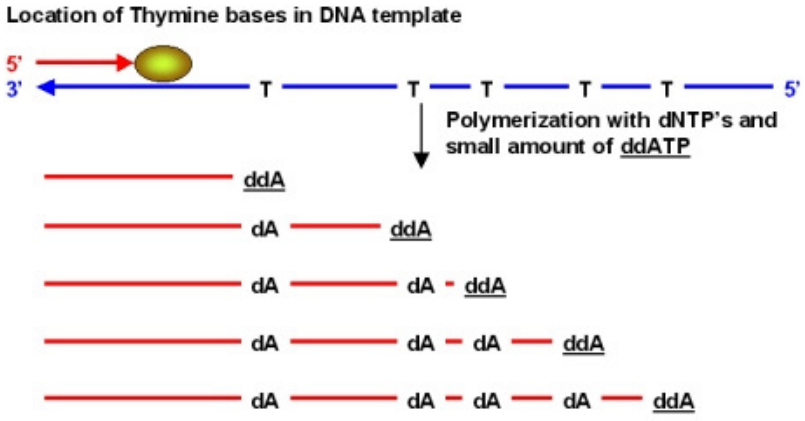


Figure 1.3- Collection of fragments of newly synthesized DNA: They all end in ddA at locations of complementary T bases in the template[3].

The newly synthesized DNA from the 4 reactions is then run (in separate lanes) on an electrophoresis gel.

- The DNA bands fall into a ladder-like sequence, spaced one base apart. The actual sequence can be read from the bottom of the gel up.
- Automated sequencers use 4 different fluorescent dyes as tags and run all 4 reactions in the same lane of the gel.
- Radioactive nucleotides (32P) are used for non-automated sequencing.
- Sequencing reactions usually produce about 500 bp of good sequence.

[3] http://www.bios.niu.edu/johns/humgen/DNA_Sequencing_and_Gene_Analysis.ppt

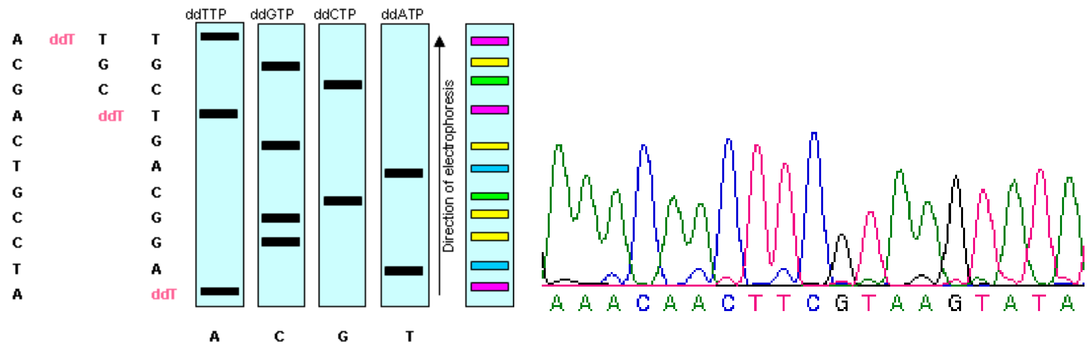


Figure 1.3- Electropherogram printout from automated sequencer for determining part of a DNA sequence.

Finally, fruit set studies are inexpensive and easy analyses, however, weather may restrict the possibilities. Monitoring pollen tube growth could make results more independent from environmental conditions and it could also accelerate the analyses. Stylar ribonuclease assay and PCR facilitate direct S-genotyping in shorter time compared to the two above mentioned methods; however they also have some shortcomings. They are more expensive and require laboratory skills and molecular biology knowledge for interpreting the results. Furthermore, PCR can be used for early selection as does not require flowering material; thereby, cost and time spent on 3–4-year-long cultivation in orchards can be saved.

CHAPTER 2:

Materials and Methods

An apricot collection orchard in region of Emilia-Romagna (Italy) belonging to the Research institute of CRPV (CENTRO RICERCHE PRODUZIONI VEGETALI) CISA, Mario Neri Consortium, was chosen to carry out the field crosses and sampling the flower shoots and young leaves for DNA extraction during the years 2008-2009 (Figure 2.1).

Figure 2.1- Apricot collection in Tebano, Experimental orchard.

2.1. Plant Materials

2.1.1 Cultivars and Accessions: Twenty four apricot varieties and accessions planted in an experimental collection orchard in Bologna province were applied to carry out the designed experiments. The list of varieties indicating their origin and available information about their pedigree is presented in table 2.1. The 5 years old trees with healthy and free of disease appearance were selected. Similar cultivation practices are applying for the trees.

Table 2.1. The list of apricot varieties, their pedigree and origin studied in this work.

	Variety	Pedigree	Origin
1	ALTERA	Harcot × (San Castrese × Reale di Imola)	Italy
2	BO92639095	Harcot × Reale di Imola	Italy
3	BO93622312	Petra Selfing	Italy
4	BORA	Early Blush × PA 7005-2	Italy
5	CORNIA	NJA1 × Bella di Imola	Italy
6	HARCOT	(Geneva Narmata) Morden 604 × NJA1 (Perfection × Phelps)	Canada
7	GHEYSI*	Unknown	Iran
8	NADERI*	Unknown	Iran
9	KIOTO	Unknown	France
10	LILLYCOT	Unknown	Canada
11	MAIA	Portici × Bora	Italy
12	NINFA	Ouardi × Tyrinthos	Spain
13	PETRA	Goldrich × Pelese di Giovanniello	Italy
14	PIEVE	Harcot × Reale d`Imola	Italy
15	PIEVE TARDIVA	Harcot × Reale d`Imola	Italy
16	PINKCOT	Unknown	Canada
17	PORTICI	Unknown	Italy
18	REALE	Unknown	Italy
19	ROBADA	Orange Red × K113-40	Italy
20	S.CASTRESE	Unknown	Italy
21	TARDIF DE VALENCE	Unknown	Spain
22	YAMAGATA 3	Unknown	Japan
23	LITO	Stark Early Orange × Tirynthos	Greece
24	GOLDRICH	Sunglo × Perfection	USA

*This cultivar iS not present in the mentioned orchard and only DNA samples have used in this work.

2.2. Pollination Tests

2.1.2 Observations in field: In order to evaluate the floral development situation of the cultivars in designed reciprocal crosses of five apricot cultivars; ROBADA, BORA, PINKCOT, MAIA and PORTICI, to consider as an important factor affecting the productivity of cultivars (fruit set percentage) and the results of pollination tests in the current experiments, during in-field studies, approximately 300 flower per cultivar were harvested to inspect the pistil situation. Referring to Faust ,1989 the size of pistils in apricot has been shown as an indicator for ability to form fruits.

Figure 2.2. the developmental situation of pistils among apricot varieties

2.2.2 Collecting Pollen - To collect the pollens for hand pollination, the shoots of different varieties containing flower buds were harvested at balloon stage or a little before and put in the plastic bags and transferred to the laboratory. The flower buds were separated and were rubbed on a suitable metal mesh with 1-2 mm holes which facilitate to separate the anthers. For each variety all the anthers were put in an aluminum dish and the dishes were labeled and exposed under white light for two days to desiccate and finally the obtained pollen grains were collected into a glass bottles and stored at -4°C until use.

2.2.3 Pollen Germination Tests - Pollen germinability was examined for seven cultivars namely; ROBADA, BORA, PINKCOT, MAIA, PORTICI, HARGRAND and GOLDRICH before hand pollination using *standard media*[4] and following the

[4] The liquid media solution contains 100.00g Sucrose, 0.02g Boric Acid and 0.3g Calcium Nitrate in one liter distilled water (pH=4.5-5.0).

procedure previously has been described as *Hanging drop Method*[5]. We proved this test to eliminate the effect of male sterility in our crosses. Then the pollen fertility was confirmed for all 7 cultivars according to the results obtained.

2.2.4 Designed Crosses - All reciprocal crosses between 5 cultivars namely: ROBADA, BORA, PINKCOT, MAIA and PORTICI were carried out. For these crosses more than 3000 flowers were emasculated and hand pollinated to evaluate fruit set to see the cross compatibility and self/non-self fertility among the cultivars. In the next year, 2009, above mentioned crosses were repeated and selfing crosses for a numerous of cultivars and accessions presented in table 2.1, grown at the same orchard was done.

2.2.5 Flower Emasculation – For controlled pollination the emasculation of the flowers is essential therefore after specifying the trees, at the correct time, the flowers at the balloon stage were emasculated by applying special scissors which cut the calyx without damaging the ovary. The late flowers and early opened ones were removed before. For each crossing the average number of emasculated flower buds was 250. After emasculation the shoots were isolated by covering them with suitable bags and the shoots maintained isolated for two weeks to avoid pollen contaminations, then the bags were taken off (Figures 2.3, 2.4 and 2.5) demonstrate the explained procedure).

2.2.6 Determining Fruit Set - During season the fruit set was investigated and the final fruit set after eight weeks was calculated *[% Fruit set = (Number of fruits *100)/Number of flowers].* As many factors in the field affect the fruit set studies, among them the developmental situation of flower buds and spring frost which damages the flowers and fruits observations was done to minimize their influence on the results for next conclusion. The pistil situation was studied according to Faust, 1989 and classified based on their size as an indicator for their ability to form fruits. The flowers with pistils smaller than 5 mms considered as undeveloped and the longer ones as developed.

[5] The simplified method to examine the germinability *in-vitro* is to dust the pollen grains on the surface of one drop from liquid media which is put on a clear glass-slide, and observing via normal microscope (x10) after 4-6 hours incubation at ambient temperature(20-22°C). The percentage of germination could be calculated by counting the number of germinated pollen grains at different microscopic views.

Figure 2.3. Apricot flowers at balloon stage , Figure 2.4. Isolating the branches after, emasculation and hand-pollination and Figure 2.5. Fruit set after eight weeks

2.3. Pollen Tube Growth Tests

2.3.1. Designed Crosses – The corresponding crosses to field reciprocal pollination were applied to follow pollen tube growth through transmitting tissue of pistils. Besides to adjust the method, the reciprocal crosses for previously genotyped cultivars GOLDRICH (S1S2) and HARGRAND (S1S2) were carried out which seems to be typically incompatible crosses.

2.3.2. Instruments and Protocols - For all 25 reciprocal crosses the shoots of selected cultivars, each containing at least 30 flowers at Balloon stage were cut and were placed in a plastic bag and transported to the laboratory immediately and placed in the room where the temperature was maintained at 20-22ºC, and the end of branches were put in beakers with 5% sucrose solution. The flowers emasculated and the next day they were ready for hand pollination corresponding to the field crosses. The branches were kept at conditions with photoperiod: 16h light/8h dark, temperature: 22-23°C and R.H.: 80-90%.

At specific time intervals; 24, 48, 72 and 96 hours after artificial pollination the pollinated pistils were harvested and placed in small glass bottles containing a 5% Formaldehyde fixing solution 40%, 5% Acetic Acid, and 90% ethanol (70%); FAA.

Table2.2. FAA mixture

Formaldehyde (40%)	Acetic Acid	Ethanol 70%
5	5	90

Table 2.3. Staining Solution; Aniline Blue 0.1%

K3PO4.3H2O	H2O	Aniline Blue(Powder)
8.8	1.0 liter	1g

To photography, an automated Nikon Eclips 90i Microscope was used. For observations the stained styles were put on clean glass slides and were covered with cover slips. The slide must be directly illuminated by ultraviolet light in a darkened room. For each cross at least five pistil samples were observed. The average length of pistils in micrometer is illustrated in table below:

Pistil	Mean	Minimum	Maximum
Length in µm	15066,33	6616,91	23275,55

2.4. DNA Analyses

2.4.1. DNA Extraction –To extract genomic DNA, the young, safe and well expanded leaves were harvested from twenty-four apricot varieties (ref. to table 2.1), in spring and the fresh leaves were conserved at -80°C before lyophilization. The Heto Drywinner model DW3 lyophilizer was used to dry freezing the leaves and 5 mg of dried leaves were put in eppendorf tube with 0.2 ml volume and were grinded in a mixture with Carbon Silicon using Mixer Mill (Retsch model MM300) with 29 rps for 3 minutes. Grinding was repeated after changing the position of the samples to get uniform grinding products. CTAB protocol was followed:

Protocol:

a) 1 ml of washing buffer (Table 2.4) was added to each tube then centrifuged with 3000 rpm for 10 minutes.

b) The supernatant was eliminated and the step using washing buffer was repeated for high polysaccharide contents.

c) For each sample 900µl of CTAB (Cetyl trimethyl ammonium bromide) restriction buffer were added. The composition of this Buffer is presented in table 2.4.

d) The pellet was solved in 0.64 ml of washing buffer and 0.16 ml NaCl 5M, 0.1 ml N-lauryl sacrosine, and 0.1 ml CTAB were added.

e) The mixture was shacked gently and incubated at 65°C for 15 minutes.

f) As the same volume as of contents, the dichlomethanol : isoamilic alcohol (24 : 1) was added to each tube and shacked very well to obtain an uniform emulsion.

g) The samples centrifuged at 1000 rpm for 5 minutes. The supernatant was transferred to a new eppendorf tubes.

h) 5 µl of RNase were added and the tubes were incubated on a shaker at 37°C for half an hour.

i) All three previous practices from adding the dichlomethanol : isoamilic alcohol (24 : 1) were repeated. Inside the tubes containing upper portion after centrifuge, 0.8 ml of cold isopropanol was added and incubated at -20°C for 15-30 minutes.

j) The tubes were centrifuged at 10000 rpm for 5 minutes and the pellets were washed with ethanol 80%. Then the pellets were dried using vacuum machine or at ambient conditions and finally the obtained DNA was dissolved in 100µl distilled water to store as stock.

Table 2.4. The solutions applied for DNA extraction

CTAB buffer	
CTAB (Sigma)	2%
NaCl	1.4 M
Tris-HCl (pH=8)	
EDTA	20mM
PVP-40	2%
β-mercaptoetanol	1%
Washing Buffer	
Ethanol	76%
Ammonium Acetate	10mM
TE solution	
Tris-HCl (pH 8)	10mM
EDTA	1mM
RNase 10mg/ml	
Dichlorometan: Isoamilic alcohol 24:1	

2.4.2. DNA Quantification and dilution – DNA concentration and quality was measured and determined as ng/µl via spectro-photomery using *NanoDrop ND-1000 UV-Vis*. The NanoDrop reports the absorbance ratio of 260/230 and 260/280 nm (wavelength), which these two last parameters present the purity of DNA from different contaminations such as protein molecules, polysaccharides and phenols. The readout will provide several important pieces of data:

- "A260" – This is the wavelength of light that is absorbed by DNA. This value is used to determine that concentration of DNA in the sample according to the conversion factor set previously (A260 of 1.0 = 50 µg ml-1 DNA).

- "A280" – The absorbance generated at 280 nm is used in the ratio A260:A280, which determines the purity of the DNA. Samples are considered of adequate purity if

A260:A280 >1.5-2, then the DNA quality is suitable for PCR analysis. After quantification tests, the DNA was diluted to obtain the standard concentration of 50ng/µl for next use.

For dilution 1:50

A/50=FD (in which A is the determined concentration of DNA obtained by NanoDrop).

100µl / FD= B µL→ the volume of DNA (stock) needed to dilute by adding the distilled water to reach to 100µl. We want a volume of 100µl of DNA.

2.4.3. Primers and PCR products- To amplify fragments of interested gene via Polymerase chain reaction the *Taq*-DNA polymerase and necessary reagents (Fisher, Invitrogen and Gold products) were used and the Termo-cycler was programmed according to the applied protocols and newly realized ones.

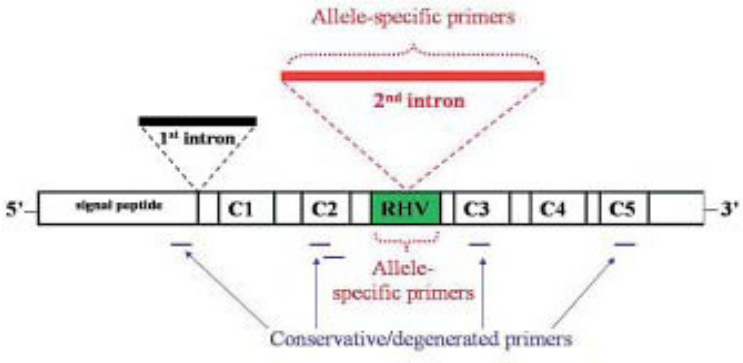


Figure 2.6- Hybridization sites of conservative and allele-specific primers designed for S-RNase based PCR.

The primer designs applied in this work include EM group primers which has designed based on analyses of twenty-two published sequences and alignments of *Prunus* S-RNases (cherry S1-S6; almond S1, S2, S7-S10, S23, Sk; Japanese apricot S1-S6; Japanese plum S1, S2) and five unpublished sequences from myrobalan (*P. cerasifera*) using CLUSTALW (DNAStar, Madison, Wisconsin, USA). Among them three degenerate primers, flanking the second intron, and based on the second, third and fifth rosaceous S-RNase conserved regions (Ushijima et al. 1998) are: EM-PC2consFD (5′-TCA-CMA-TYC-ATG-GCC-TAT-GG-3′), EM-PC3consRD (5′-

AWS-TRC-CRT-GYT-TGT-TCC-ATT-C-3′) and EM-PC5consRD (5′-CAA-AAT-ACC-ACT-TCA-TGT-AA-CAR-C-3′). These EM primers were used in C2 + C3 and C2 + C5 combinations. The primers SRc-F(5′-CTCGCTTTCCTTGTTCTTGC-3′)(Romero et al.2004) and SRc-R, 5′-GGCCATTGTTGCACAAATTG-3′(Vilanova, 2005) are designed from conserved region of *P.armeniaca* S-RNase genomic sequence. The primers SFB have designed from the consensus sequence of *SFB* alleles identified in *Prunus dulcis* (Ushijima et al., 2003), Prunus mume (Entani et al., 2003), and *Prunus armeniaca* (Romero et al., 2004 and Vilanova et l., 2006), (Table 2.6).

The set of PaCons primers have designed from three regions that were highly conserved among the six alleles, the signal peptide C2 and C5 (Figure 2.7). The first pair, PaConsI-F and PaConsI-R, was applied to amplify the first intron, located between the regions coding for the signal peptide region and the mature protein. The second pair, PaConsII-F and PaConsII-R, was applied to amplify the second intron, located in the RHV region between C2 and C3. The primers; SFB and SFBc-R; which have generated according to the alignment of European apricot SFBs S1(AY587563), S2(AY587562) and S4(AY587565) (Romero et al. 2004), were applied to identify eight F-box haplotypes (alleles).

Table 2.5. Primers applied to amplify *S*-locus in apricot

	Primer	Sequence(5'-3')	Reference
1	EM-PC2consFD	TCACMATYCATGGCCTATGG	Sutherland 2004
2	EM-PC3consRD	AWSTRCCRTGYTTGTTCCATTC	Sutherland 2004
3	EM-PC5consRD	CAAAATACCACTTCATGTAACARC	Sutherland 2004
4	SRc-F	CTCGCTTTCCTTGTTCTTGC	Romero 2004
5	SRc-R	GGCCATTGTTGCACAAATTG	Vilanova 2005
6	SFBc-F	TCGACATCCTAGTAAGACTACCTGC	Vilanova 2006
7	SFBc-R	ATTTCTTCACTGCCTGAATCG	Vilanova 2006
8	SFBa-1R	TAAACCTCAACCGCCAAGAC	Romero 2004
9	SFB-5F	TAGGACCCCTCCAATGAGC	Romero 2004
10	SFB-6F	TGGGTTCTGCAAGAGAAACG	Romero 2004
11	PaConsI-F	(C/A)CTTGTTCTTG(C/G)TTT(T/C)GCTTTCTTC	Sonneveld 2003
12	PaConsI-R	CATG(A/G)ATGGTGAA(A/G)T(T/A)TTGTAATGG	Sonneveld 2003
13	PaConsII-F	GGCCAAGTAATTATTCAAACC	Sonneveld 2003
14	PaConsII-R	CA(T/A)AACAAA(A/G)TACCACTTCATGTAAC	Sonneveld 2003

Table 2.6 . PCR Mixture (standard)	
Bi-Distilled sterilized H2O	15.8 µl
Reaction Buffer	2.0 µl
MgCl2 25 mM	0.5 µl
dNTPs 10mM	0.2 µl
Primer Forward 10µM	0.2 µl
Primer Revere 10 µM	0.2 µl
Taq-DNA polymerase 5U/µl	0.1 µl
DNA 50 ng/µl	1.0 µl

Table 2.7. Standard PCR protocol

Phase	Temp.(°C)	Time	Cycle
Initial denaturation	94	3′	1
Denaturation	94	30″	32
Annealing	58	45″	
Extension	72	1′	
Final extension	72	10′	1

The agarose gel with 1.5 to 2% was used during identification of amplified fragments belonging to the 1st and/or 2nd intron of locus S of the studied varieties. Usually this separation technique is useful for the separation of fragments bigger than 300bp; otherwise the Polyacrylamide gel was prepared as explained later.

To prepare the agarose gel the following protocol was applied:

Protocol:

a) 1.5g agarose were dissolved in 150ml TAE 1X by heating,
b) The solution was left to get cool to ~50°C before purring into the matrix,
c) The suitable combs were inserted,
d) After 20 min. the gel was ready to use.

Table 2.8. Solution for electrophoresis on agarose gel

TAE buffer 50×	
Tris Base	242 g
Acetic Acid (glacial)	57.1 ml
EDTA 0.5 M, pH8	100 ml
Distilled H2O	To reach vol. 1 l
Blue	
TAE 50×	2 ml
Ficoll 400	2.5 g
Bromophenol Blue	25 mg
Distilled H2O	To reach vol. 20 ml

After running the staining was carried out according to the table .The gel was put in the staining solution for 15-20 minutes and was observed via Kodak Image Station 440 CK and analyzed using Kodak ID software.

Table 2.9. Staining solurtion

Ethidium Bromide	100 µl
Distilled H2O	1 l

The Polyacrylamide gel 5% was applied when using SSR marker to evaluate the polymorphism in locus S and when the doubtful results were obtained in S-genotyping procedure of studied varieties which were due to the small amplified fragments. To prepare the gel the following protocol was applied:

The gel had to be prepared at least 2 hours before use.

Protocol (referring to table 2.10 and 2.11):

a) First polishing the correct side of both glasses (Repel and Bind) via treatment by Ethanol 100% and 40% respectively.
b) The bind glass had to be treated with bind solution(containing ethanol 95%, acetic acid 0.5% with 3µl γ-metacryloxypropyl-trimthoxysilane or bind silane.
c) The Repel will treat by 800µl of ‘‘SigmaCote’’
d) After 5 minutes both surfaces had to polish with Ethanol 100% and then 40% respectively.
e) Put on the borders of one of the glasses considering the treated surface, the specific combs to make a 0.3mm space between two glasses after putting the other over the first one. The two combined glasses had to be stabilized using clamps.
f) The Polyacrylamide gel (14%) had to be injected into the space from the solely opening board and then should be covered using a nylon foil.
g) The next day, the gel is ready to mountain on electrophoresis machine inside the TBE 1X.
h) 4-5µl of each sample were injected into wells and they left to run for 3-4h., applying 65 Watt electric power.
i) At the end of electrophoretic migration, put the gel in Fix solution(table) for 30 min.
j) Wash 2 times each 5 minutes in bi-distilled water.
k) Put the gel in staining solution(table) for 30 min. on shaker.
l) Put the gel into the develop solution(table) till the bands appear.

Table 2.10. Solutions applied for Polyacrylamide gel electrophoresis

TBE buffer 5×	
Tris Base	54 g
Boric Acid	27.5 g
EDTA 0.5 M, pH 8	20 ml
Distilled H2O	To Vol. 1 l
TBE – Urea	
Urea	210 g
TBE 5×	100 ml
Distilled H2O	To vol. 500 ml
Polyacrylamide gel 5%	
TBE – Urea	54ml
Acrylamide solution 40%	8 µl
Bis-acrylamide solution	8 µl
TEMED	45 µl
APS 10% solution	300µl
Denaturant reagent	
EDTA 0.5 M pH 8	0.5 ml
Blu di bromofenolo	25 mg
Xilene cianolo	25 mg
Formamide 98%	To vol. 25 ml

Table 2.11. Solutions for fixing and staining of polyacrylamid gel

Fix Solution	
Acetic acid 10% v/v in distilled H2O	
Staining solution	
AgNO3	1 g
Formaldehyde 37%	1.5 ml
Bi-distilled H2O	To vol. 1 l
Developing solution	
Na2CO3	60 g
Formaldehyde 37%	3 ml
Sodium Tiosulphate 1%	400 µl
Bi-distilled H2O	To vol. 2 l

2.4.3.1. Amplifying S-RNase Gene Fragments- The previously designed and applied primes for other *Prunus* members were used to amplify the fragments of S-RNase gene involving 1st and/or 2nd intron. The list of the applied primers and their sequences are presented in Table (2.6).

Table 2.12. SRC mix solution and obtimized SRc-PCR program∗

	1X(µl	25X(µl)
Water	18.375	459.375
Buffer	2.5	62.5
MgCl2	1	25
dNTPs	0.4	10
Primer F	0.8	20
Primer R	0.8	20
Taq-Polymerase	0.125	3.125
DNA	1	xx
Total	25	600

94°C	1 min	Initial Denaturation	94°C	1 min	Initial Denaturation
94°C	10"	37 times	94°C	20"	37 times
60°C	1 min		60°C	50"	
68°C	3 min		68°C	1 min	
68°C	10 min	Final Extension	68°C	10 min	Final Extension

*The program on left is modified and optimized one which applied in this work.

Table 2.13. PaCons I,II mix solution and obtimized PaCons-PCR program

	1X(µl)	25X(µl)
Water	12.675	316.875
Buffer	2	50
MgCl2	0.7	17.5
dNTPs	0.5	12.5
Primer F	1	25
Primer R	1	25
Taq-Polymerase	0.125	3.125
DNA	2	
Total	20	450

95°C	2 min	Initial Denaturation	
94°C	30"	minus 0.5°C /cycle	10 times
60°C	1 min		
68°C	2.5 min		
94°C	30"		25 times
55°C	1 min		
68°C	2.5 min		
68°C	10 min	Final Extension	

Table 2.14. EMPC mix solution and EMPC-PCR program

	1X(µl)	25X(µl)
Water	13.775	344.375
Buffer	2	50
MgCl2	1.2	30
dNTPs	0.5	12.5
Primer F	0.7	17.5
Primer R	0.7	17.5
Taq-Polymerase	0.125	3.125
DNA	1	xx
Total vol. (µl)	20	475

94°C	Initial Denaturation
94°C	35 times
58°C	
68°C	
68°C	Final Extension

Table 2.15. Multiplex* (SRc/EmpC)

	1X(µl)	25X(µl)
Water	16.175	404.375
Buffer	2.5	62.5
MgCl2	1.5	37.5
dNTPs	0.6	15
Primer F	1.5	37.5
Primer R	0.8	20
Prime R2	0.8	20
Taq-polymerase	0.125	3.125
DNA	1	xx
Total vol. (µl)	25	600

Table 2.16. SFB and SFBc mix solution and SFB/SFBc-PCR program

	1X(µl)	25x(µl)
Water	14.175	354.375
Buffer	2	50
MgCl2	0.7	17.5
dNTPs	0.4	10
Primer F	0.8	20
Primer R	0.8	20
Taq-Polymerase	0.125	3.125
DNA	1	xx
Total vol. (µl)	20	475

94°C	2 min	Initial Denaturation
94°C	30"	30 Times
55°C	1 min	
2°C	1.5 min	
72°C	10 min	Final Extension

2.4.3.2. Amplifying SFB Gene Fragments- The mixture and PCR program illustrated in Table 2.16. were applied. Two sets of primers: SFB and SFBc were used with the same PCR protocol. Although they were able to amplify the desired fragments among our varieties but to discriminate these fragments we need to apply a technique other than agarose gel electrophoresis.

2.4.3.3. Single Strand Conformation Polymorphism (SSCP)- This technique which works based on the nucleotide sequence variation among the DNA single strands, for the first time was applied in human genetic studies in 1993 and recently has applied in plants. The limitation of using this technique refers to the primers which should be specific for each locus. For this it is necessary to design primers based on the gene sequence. Besides, another limitation is the size of fragments that seems to give the doubtful results for large sized fragments.

The PCR was conducted for characterization of *SFB* variants. The Master Mixture was prepared as table and table and divided in tubes. The amplification was done according the program in table 2.17. The amplification was validated via running the products on agarose gel 2%. To prepare the PCR products for SSCP analysis, to the 2 µl of the product, 9 µl of denaturant solution were added and denaturized for 10 min. at 95°C. Then the product was transferred into ice containing box. The specific Polyacrylamide gel was prepared according to following protocol:

Protocol (referring to Table 2.17.)**:**

a) First polishing the correct side of both glasses (Repel and Bind) via treatment by Ethanol 100% and 40% respectively.
b) The bind had to be treated with bind solution containing γ-metacryloxypropyl-trimthoxysilane or bind silane.
c) The Repel will be treated by 800µl of ‘‘SigmaCote’’
d) After 5 minutes both surfaces had to be polished with Ethanol 100%.
e) Putting on the borders of one of the glasses considering the treated surface, the specific combs to make a 0.7mm space between two glasses after putting the other over the first one. The two combined glasses had to be stabilized using clamps and then with agarose 1% to seam up all around of the glasses.
f) The MDE gel (corresponding to 14% normal Polyacrylamide gel) as illustrated in table 2.17 had to be injected into the space from the solely opening board and then should be covered using a nylon foil.
g) The next day, the gel is ready to mountain on electrophoresis machine inside the TBE 0.6%.
h) 4µl of each sample would be injected into wells and they leaving to run overnight or 15-16h applying 2W electric power.
i) Putting the gel in Fix solution for 3 min.
j) Putting the gel in staining solution for 5 min.
k) washing the gel in distilled water.
l) Putting the gel into the develop solution till the bands appear.

Table 2.17- Solution applied for MDE gel preparation and staining

MDE solution			
Reagent	In 20 ml solution	In 25 ml solution	In 30 ml solution
Glycerol 50%	2 ml	2.5 ml	3 ml
MDE	5 ml	6.5 ml	8.5 ml
TBE 5×	2.4 ml	3 ml	4 ml
H2O	10.6 ml	13 ml	14.5 ml
APS	100 µl	125 µl	150 µ
TEMED	12.5 µl	15 µl	17 µl
Denaturant solution			
Formamide 100% (95%)	950 µl		
NaOH 1M (0.01M)	5 µl		
Xylene cianolo 5% (0.05%)	5 µl		
Blu di bromophenol (0.05%)	10 µl		
Binder (41ml) (γ-methacryloxypropyl-trimethoxysilane/bind silane			
Ethyl alcohol 100%	40 ml		
Acetic acid 10%	1.2 ml		
Bind	60 µl		
TBE 10× (1000 ml)			
Tris	108 g		
Boric acid	55 g		
EDTA	40 ml		
H2O	To vol. 1 l		
Fix solution (200 ml)			
Ethyl alcohol 100%	20 ml		
Acetic acid 100%	1 ml		
H2O	179 ml		
Staining solution			
AgNO3	0.2 g		
Fix solution	100 ml		
Develop solution			
NaOH	3 g		
Formaldehyde	270 µl		
H2O	To vol. 100 ml		

2.5. Cloning

2.5.1 Preparing plasmid recombination- The pGEM-T vector system, Promega kit was applied for recombination via ligation reaction following protocol presented in table 2.19.

Table 2.18. Ligation protocol

PCR product	3 µl
pGEM-T plasmid	1 µl
Ligase ATP 2×	5 µl
T4 DNA Ligase	1 µ

The mixture was incubated overnight at 4°C.

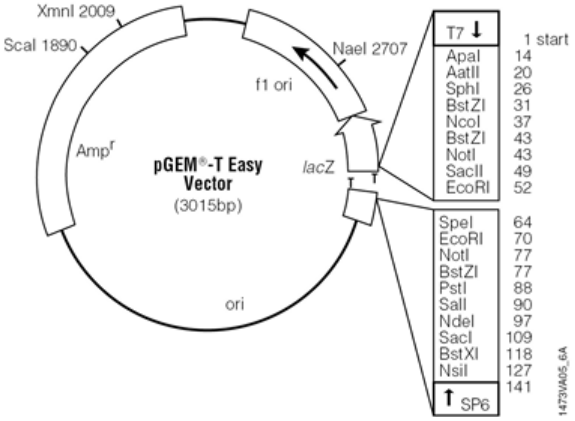


Figure 2.8- The map of pGEM- T Easy vector

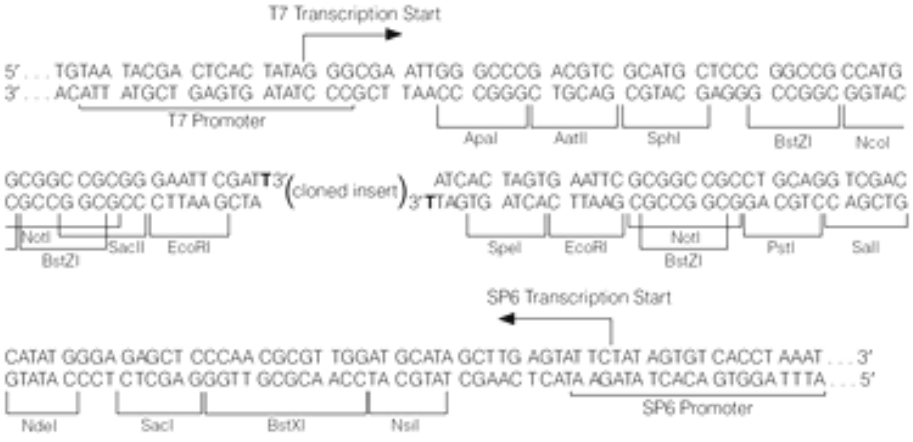


Figure 2.9 – Multiple cloning region of pGEM- T Easy vector

2.5.2 Preparing the culture media- The LB (*Luria and Bertani*) is commonly applied to culture the *Escherichia coli,* during cloning. To do this procedure the media was prepared according to the table 2.20 and then agar was added to solidify it. Then autoclaved and after getting cool (~50°C) ampicilin(50µg) was added. And divided into sterile Petri dishes. At the time of use, 40 µl X-Gal and 4 µl IPTG solution were distributed on the media surface.

2.5.3 Transformation-The desired fragments were inserted in the *Escherichia coli* (DH5α) as following protocol:

Protocol:

a) The Competent cells were put in ice after taking them out of the freezer -80°C
b) In the 13 ml vol. propylene tubes 0.1 ml of the competent cells suspension were injected and 2µl of ligation product were added. The tubes were incubated at 37°C on the shaker.
c) For thermal shock, the tubes were soaked in the bath at 42°C for 45". Then immediately were put in the ice for couple of minutes.
d) 900 µl of media SOC were added to each tube and the tubes were incubated for 1h. at 37°C to allow multiplication of Competent cells.
e) 100 µl of the suspension were distributed on the solid LB+ampicilin+IPTG+XGal media in Petri dishes and incubated at 37°C for 16-24h.

Table 2.20. Culture medias for cloning

Culture media LB	
Bacto – Tryptone	10 g/l
Bacto – Yeast extract	5 g/l
NaCl	5 g/l
Culture media 2XYT	
Bacto – Tryptone	16 g/l
Bacto – Yeast extract	10 g/l
NaCl	5 g/l
Culture media SOC	
Bacto – Tryptone	2 g/100ml
Bacto – Yeast extract	0.5 g/100ml
NaCl 1M	1 ml/100ml
KCl 1M	0.25 ml/100ml
MgCl2 2M	1 ml/100ml
Glucose 2M	

2.5.4 Isolation of transformed colonies- The ampicilin applied in media make possible to recognize the transformed cells versus non-transformed ones via their appearance. In fact the X-Gal and IPTG allow the recognition of the transformed cells with plasmids containing the desired DNA fragments versus the transformed cells with plasmids non-containing the desired fragments. The vector pGEM contains a sequence which codifies the β-galactosidase enzyme which in the presence of X-Gal produce blue colored product. The IPTG induces transcription of the enzyme. According to this mechanism, the white colonies were selected as transformed ones for recombinant DNA extraction. At least 8 single colonies for each sample were isolated and cultured on the LB+ampicilin media in Petri dishes and incubated at 37°C overnight to manipulate the colonies. To confirm the transformation products, the colony-PCR was carried out based on the previously described master mix preparations and PCR programs. In the case of S-RNase based genotyping the agarose gel 2% electrophoresis was applied. For F-box based genotyping the SSCP technique was performed.

To conserve the identified allelic variant colonies, the glycerol stock was prepared. 1ml of LB + ampicilin was put into the 2ml ependorf tubes and were infected by selected colony and the tubes were incubated at 37°C overnight and then 0.5ml of sterile glycerol 60% was added to each tube. The samples were stored at -80°C in freezer until use.

2.5.5 Plasmid –DNA extraction and quantification- the following protocol was applied to extract plasmid-DNA:

Protocol (Referring to table 2.21)**:**

a) The selected colony was multiplied using 5ml liquid LB+5µl ampicillin in 13ml propylene tubes incubating at 37°C overnight
b) The suspension was centrifuged at 1500-3000 rpm for 5min., removing the supernatant completely. The concentrated portion was transformed into a 1.5ml eppendorf tube.
c) The tube was centrifuged at 3000 rpm for 10min and all remained culture media was removed.
d) The pellet was dissolved in 0.2ml of solution A(table), and was put in ice.
e) 0.4ml of solution SDS were added and the tube left in ice for 5min.
f) 0.3ml of KOAc 3M were added and the tube was shacked gently.
g) The contains of tube were centrifuged at maximum for 10min.
h) 0.85ml of the supernatant n was transferred to a new tube and 0.6ml cold isopropanol was added and mixed very well and left in freezer for 10min to speed up cooling.
i) The tube was centrifuged at maximum speed for 20 min.
j) The supernatant was removed and the pellet was washed in 1ml washing buffer(ethanol 70%)
k) The pellet left to dry at ambient temperature and then was dissolved in 50µl distilled sterile water. The tubes were incubated at 65°C for 10min.

Table 2.21. Solutions and chemicals for plasmid-DNA extraction

Solution A	
Glucose	10% w/v
EDTA, pH 8	10 mM
Tris, pH 8	25 mM
Alkalin SDS	
NaOH	0.1 M
SDS	1% w/v
KOAc 5M pH 4.8	
Acetate Potassium	5 M
Acetic Acid glacial	0.23% v/v

2.5.6 Sequencing- To preparing the plasmid-DNA samples for sequencing the quantity and quality of extracted DNA were determined as previously description. And finally were diluted according to the determined concentrations (explained in part 2.4.2. of this chapter.For sequencing we applied on-line to

http://www.biofabresearch.it sending the samples to Bio-Fab research Center in Rome, Italy.

2.5.7 Data Analysis- The following bioinformatic programs were applied to analyze the data:

- Reverse complement of sequence; http://searchlauncher.bcm.tmc.edu/seq-ptions/revcomp.html
- EMBL Nucleotide Sequence Database Collaboration EBI.url; http://www.ebi.ac.uk/embl/contact/collaboration.html
- pGEM-T vector:Sequence; http://www.promega.com/vectors/pgemt.txt
- Homology Analysis: FASTA, http://www.ebi.ac.uk/Tools/fasta33/index.html
- CLUSTALW WWW System; http://clustalw.ddbj.nig.ac.jp/top-e.html
- FASTA WWW System; http://fasta. ddbj.nig.ac.jp/top-e.html
- Sequence alignment analysis, http://www.ebi.ac.uk/Tools/clustalw2/index.html
- Chromas LITE version 2.01 available on-line: www.technelysium.com.au

Chapter 3:

Results and discussions

3.1. Results:

3.1.1. Fruit Set Studies-The results of field observations are presented in the following parts including flower morphology notes, field crosses and laboratory controlled pollinations.

As mentioned in previous chapter, to clarify the floral development situation of the cultivars studied for compatibility, an observation was done via flower bud sampling and pistil inspection to determine their ability to form fruits referring to the size of pistils. The following Table (3.1) and histogram (figure 3.1) show the results obtained in 2008 and 2009.

Table 3.1-The variability of five apricot pistils developmental situation observed by sampling more than 2400 flowers within two years

Year	Cultivar	Pistil abscent	Short (<5mm)	Medium (5-10mm)	Long (>10mm)	Brown Ovary	Total
2008	ROBADA	5	33	15	49	248	350
	BORA	9	3	50	99	44	205
	PINKCOT	22	95	24	110	100	351
	MAIA	20	71	15	94	152	352
	PORTICI	29	16	28	196	83	352
	Total	85	218	132	548	627	1610
	Average	17	43.6	26.4	109.6	125.4	
2009	ROBADA	4	38	46	47	19	154
	BORA	0	83	28	15	31	157
	PINKCOT	0	32	25	99	4	160
	MAIA	7	113	11	16	31	178
	PORTICI	0	35	10	111	2	158
	Total	11	301	120	288	87	807
	Average	2.2	60.2	24	57.6	17.4	

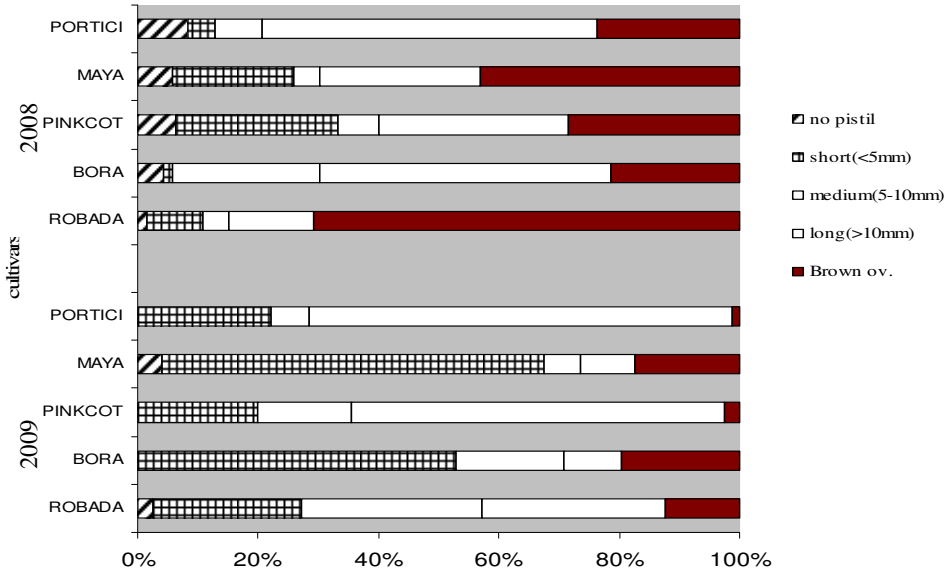


Figure 3.1 - The pistils developmental situation among the studied cultivars in 2008 and 2009.

Considering that the pistils with size from 5 mm to >10 mm are able to set fruits after pollination (naturally or artificially), the behavior of cultivars is different, also for the same cultivar the situation differs year to year. Although in this study the biological aspects of fertility are not the aim, but it can not be neglected that these parameters are affecting the fruit set determination. During emasculation activity we tried to use the best flowers, but in some cases the tree by nature produce small-sized pistils, which will affect the fruit set results. Moreover, other environmental conditions such as frost damage also are interfering fruit set studies. According to results PORTICI and PINKCOT are more stable cultivars for this trait and opposite to them ROBADA and BORA, are variable cultivars. Producing flower without pistils seems to be a dependent trait to environment condition, and brown ovaries, too, as could be concluded from presented data.

For designed crosses the pollens were collected for hand pollination and before using them, their ability to germinate was tested as previously described in chapter 2. The results indicated the acceptable vitality and germinability (Table 3.2). The germination test was carried out only for 7 cultivars in this experiment as the reciprocal crosses were designed for these cultivars. Actually, it has been found when studying pollen from apricot cultivars that with the exception of some male sterile cultivars most of the apricot cultivars produce pollen in quantities with a high percentage of viability and

germinates, emitting a pollen tube, in a wide range of temperatures (Vachun, 1981; Egea et al., 1992).

Table 3.2 -Pollen Germination test* after 4 h. At 22-23 C

Cultivar	Average
ROBADA	48.8%
BORA	47.25%
PINKCOT	59.1%
MAIA	73.37%
PORTICI	57.75%
GOLDRICH	76%
HARGRAND	21%
* Mean of 3 replications calculated as percentage	

The low percentage obtained for cultivar HARGRAND is probably due to the flowers developmental stage during pollen collection.

In 2008 the reciprocal crosses were carried out for 5 cultivars. The final fruit set determined after 8 weeks and the results are illustrated in Table 3.3. The fruit set was monitored during season and traced until harvesting time, but as in apricot, the partenocarpy never has been reported, therefore waiting to harvest fruits to evaluate the fruit set is not necessary. But the fruits harvested at ripening time, then the embryos can be use for later studies to evaluate the trait segregation. Referring to the Tables 3.3 and 3.4, indicating the results of self and cross pollination tests obtained during the years 2008 and 2009, the self-incompatibility of cultivars ROBADA, PINKCOT, GOLDRICH and HARGRAND is confirmed.

Some apparently contradictory results can be due to the environmental (weather condition), such as frost damage of fruit primordia that happened in spring 2008, and physiological aspects of flower development, for these reasons the crosses between ROBADA × PORTICI or BORA × MAIA have significantly different fruit set in two years. The crosses between PINKCOT as Female and other four cultivars is relatively low, although the percentage of fruit set in 2009 shows cross compatibility.

Several factors are influencing the fruit set which requires non-negligible attention to the results. During emasculation we tried to maintain the flowers with pistils bigger

than 10 mm (eye sized), in the case of cultivar ROBADA, the large number of pistils were Undeveloped (Figure 3.1).

Table 3.3 - Fruit set (%)

Female Parent	Male Parent	Year 2008	Year 2009	Notes
ROBADA	ROBADA	0	0.8	Self-incompatible
	BORA	8.9	21.4	Cross compatible
	PINKCOT	8.5	19.2	Cross compatible
	MAIA	9.5	25.3	Cross compatible
	PORTICI	2	27.7	Cross compatible
BORA	BORA	8.8	13	Self-compatible
	ROBADA	18	19.1	Cross compatible
	PINKCOT	10.6	19.5	Cross compatible
	MAIA	3.1	17.6	Cross compatible
	PORTICI	14.4	28.7	Cross compatible
PINKCOT	PINKCOT	0	6	Self-incompatible
	ROBADA	1.5	6	Cross compatible
	BORA	3	10.5	Cross compatible
	MAIA	0	6	Cross compatible
	PORTICI	0	9.1	Cross compatible
MAIA	MAIA	0	Fruitful	Self-compatible
	ROBADA	2.4	No data	Cross compatible
	BORA	7	No data	Cross compatible
	PINKCOT	5.2	No data	Cross compatible
	PORTICI	0.5	No data	Cross compatible
PORTICI	PORTICI	12.2	20	Self-compatible
	ROBADA	21.1	23	Cross compatible
	BORA	19.3	23.1	Cross compatible
	PINKCOT	20.4	27.8	Cross compatible
	MAIA	19.9	20.8	Cross compatible
Average number of hand pollinated flowers/bag>250				

Also, for the cultivar MAIA, the situation was similar. Thus the poor fruit set results obtained for cultivar MAIA as female parent might be due to this reason and not specifically for incompatibility trait. Among the observed cultivars it seems that the cultivar PORTICI is the most reliable parent as pollen recipient and donor. One

logical reason for good fruit set recorded in 2009 with the same crosses, is the satisfactory of environmental condition comparing with the year 2008.

Table 3.4. Fruit set (%) obtained from self-pollination program in 2009 for available number of apricot varieties at Tebano Reserch Station

Cultivar/acc.	2009	Note
PIEVE	10	Self-compatible
ROBADA	2	Self-incompatible
BORA	14	Self-compatible
PINKCOT	0	Self-incompatible
MAIA (MAYA)	No data	Self-compatible*
S.CASTRESE	30	Self-compatible
KYOTO (KIOTO)	42.5	Self-compatible
PORTICI	14,5	Self-compatible
BO93623012	6.5	Self-compatible
BO92639095	10	Self-compatible
T.DE VALENCE	29	Self-compatible
REALE	No data	Self-compatible*
PETRA	8	Self-compatible
PEIEVE TARDIVA	15	Self-compatible
HARCOT	SI	Self-incompatible
LITO	No data	Self-compatible*
GOLDRICH	0	Self-incompatible
HARGRAND	0	Self-incompatible
Average number of hand pollinated flowers/bag ~150 * REALE DI IMOLA and LITO are known to be self-compatible. MAYA evaluated by another work in the same year and showed self-compatibility.		

Any way the complications in understanding the effects of numerous factors affecting classical methods of these kinds of studies forced the breeding researchers to find more controllable methods of work such as following pollen tube growth by optical microscopy.

3.1.2. Following pollen tube growth-. Along the style the pollen tube growth was monitored from germination point on the stigma toward ovary. The results obtained by examining 5 pistil samples for each cross A combination was considered compatible when at least one pollen tube succeeded in reaching the ovary within 72 hours after pollination. In contrast, if pollen tube growth was arrested and terminal callus plugs were seen to form, the combination was considered incompatible. At specific time intervals; 24, 48 and 72 hours after artificial pollination the pollen tube growth were examined and it was found that after ≥72h almost in all apricot cultivars the pollen tube can reach to the ovary in the case of compatibility. For this reason the reports are demonstrated here for 72h after hand pollinating. The results are summarized in Table 3.5. Although Fixing pollen tube growth 96 h after pollination leaves enough time for tubes to reach the ovary in case of apricot (Viti et al., 1997; Audergon et al., 1999), but as we proved during first year of our experiments, it was found that inspecting pistils after 72h seems to be sufficient for apricot varieties studied here, except for PORTICI combinations as female parents, pollen tubes need more time to reach exactly inside the ovary of compatible combinations, but enough to verify the compatibility/incompatibility reactions. The results obtained are confirming the field test crosses. Among the cultivars studied here no cross incompatibility was identified except HARGRAND (*S1S2)* × GOLDRICH (*S1S2)* crossing, which was used as negative control of analyzing compatibility by microscopy. Although semi/partial fertility results in poor productivity of some cultivars but with field cross tests it does not seem to be easy to understand. The fate of crosses is mentioned in description column of the Table 3.5. The micrography was done and the photos are illustrated (Figures 3.2a to 3.2e). The details are described for each figure. The photos are illustrating the whole view of pistils from stigma to ovary, to facilitate following the pollen tube growth (RO=ROBADA, BO=BORA, PINK=PINKCOT, MA=MAIA, PO=PORTICI, GR=GOLDRICH and HAR=HARGRAND). The pollens arrested at the one-third of their pathway toward ovary considered to match with one of the alleles of pistil genotype.

Table 3.5 - Following pollen tubes in planned artificial crosses under controlled condition

Female (Pollinated pistil) After 72 hours	Male (Pollen)	Pistil No.	Pollen on Stigma	Germinated Pollens (N,L,M,H)•	Pollen tube In ¼ style	Pollen tube in ½ style	Pollen tube in ¾ style	Pollen tube in basal point	Pollen tube in ovary	Description: •Notes: N: no germination L: low germination (5-10) M: medium germination (20-40) H: high germination (>50)
ROBADA *(S8S22)*	ROBADA	3	X	H	X			X*		Many of pollens are arrested by calluse formation at the tip. Abnormality can be seen in pollen tubes growth. *Rare number of pollen tubes have continous growth toward ovary which is a result of pollen contamination during hand pollination.
	BORA	4	X	M-H					X	In all four samples the tubes are introduced to the ovary and may be inside the micropole.The cross is completely compatible.
	PINKCOT	3	X	L-M		X			X	Compatible Cross
	MAIA	1	X	L			X			The pistils are not developed perfectly. They are very short.
	PORTICI	3	X	L					X	Compatible cross
BORA *(ScS9)*	ROBADA	3	X	M-H					X	Compatible cross
	BORA	2	X	H					X	Compatible cross
	PINKCOT	2	X	M-H					XX	Compatible cross, (fertilized!)
	MAIA	2	X	M				X	X	The stigma is damaged
PINKCOT *(S8S9)*	ROBADA	7	X	M	X	X			X	2 pollen tubes in ovary. Very short pistils
	BORA	5	X	M-H					X	15 Pollen tubes in ovary
	PINKCOT	5	X	H	X					All pollen tubes are arrested at the 1/3 of style.
	MAIA	6	X	H	X		X		X	12 pollen tubes observed in ovary
	PORTICI	6	X	H					X	29 pollen tubes in the ovary
PORTICI *(S2S17)*	ROBADA	5	X	M-H					X	6 Pollen tubes in the ovary
	BORA	5	X	H					X	7 pollen tube in the ovary
	PINKCOT	5	X	H				X	X	6 pollen tube in the ovary
	MAIA	5	X	M-H					X	7 pollen tube in the ovary
	PORTICI	5	X	H					X	6 pollen tubes in the ovary
MAIA *(S2S9)*	ROBADA	5	X	H					X	13 pollen tubes in the ovary
	BORA	5	X	H	X				X	29 pollen tubes in the ovary
	PINKCOT	5	X	H				X	X	8 pollen tubes in the ovary
	MAIA	5	X	M			X		X	9 pollen tubes in the ovary
	PORTICI	5	X	H					X	5 pollen tube in the ovary

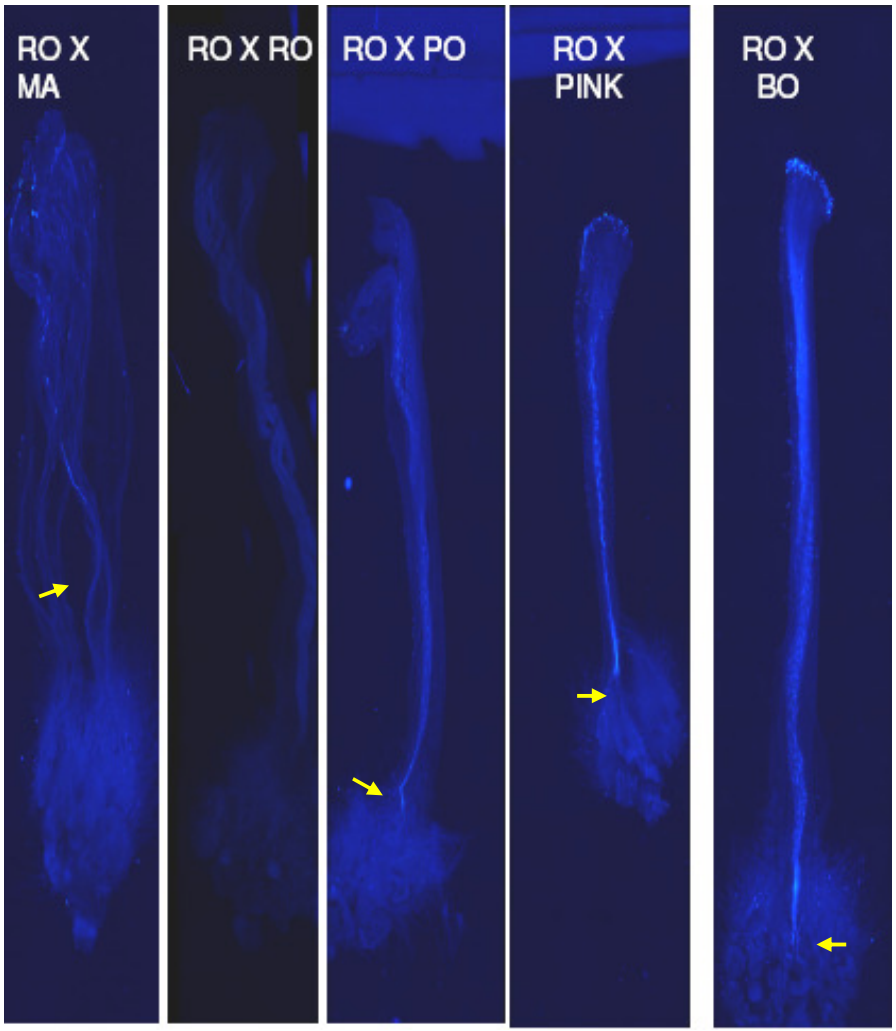


Figure 3.2a.

The microscopic photos are presenting the pistil of ROBADA *(S8S22)* how interacts with the pollen of other four cultivars 72 hours after hand pollination . From left to right the pollen tubes of MAYA (*S9S2*) were followed clearly till the basal point. The next photo of ROBADA selfing shows the incompatibility after self pollinating as no pollen tubes were seen along the pistil. We find growing pollen tubes after very short elongation reaching to one-third of style length. The self-incompatibility of ROBADA is confirmed. The third photo from left indicates clearly the presence of pollen tubes in the ovary which means that pollens of PORTICI with *S2* and *S17´*genotype are able to grow towards ovary. Also the next two crossings; ROBADA×PINKCOT and ROBADA×PORTICI demonstrate the pollen tubes reaching the ovary of ROBADA as all these crossings have at least one allele not in share. (RO=ROBADA, BO=BORA, PINK=PINKCOT, MA=MAIA, PO=PORTICI).

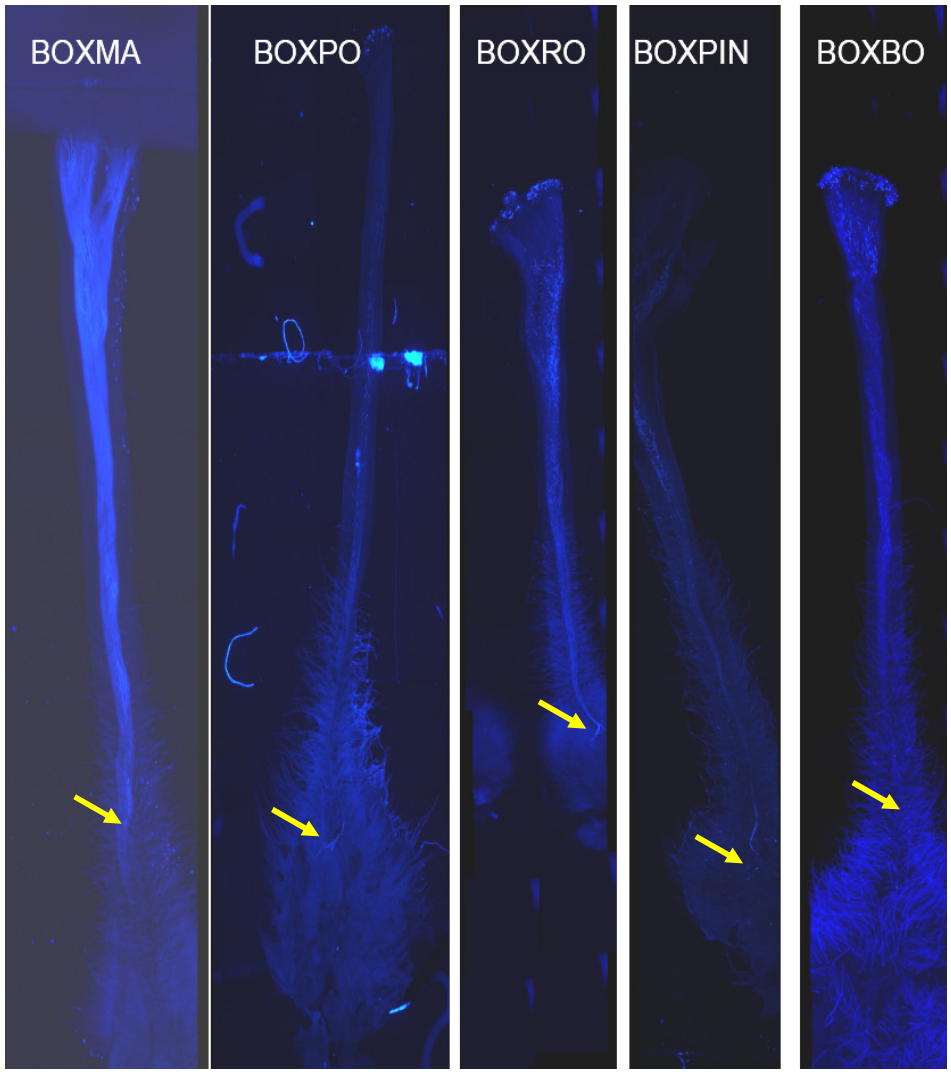


Figure 3.2b.

The hand pollinated pistils of BORA after 72 h at controlled conditions all are indicating the compatibility of pollens . The tubes are reaching into the ovary or basal point of pistils confirming the cross compatibility between BORA (*ScS9*) as female parent and ROBADA (*S8S22*), MAYA (*S9S17'*), PINKCOT (S8S9) and PORTICI (*S2S17'*). Besides the self-compatibility of this cultivar is approved, too. The arrested pollen tubes with the same s-genotype at the upper one-third length of pistils could be seen. all these tubes are stopped by callose deposition in the tip of them. The pollens germination on the stigma is considerable indicating that the pollen quality and germinability are perfect(RO=ROBADA, BO=BORA, PINK=PINKCOT, MA=MAIA).

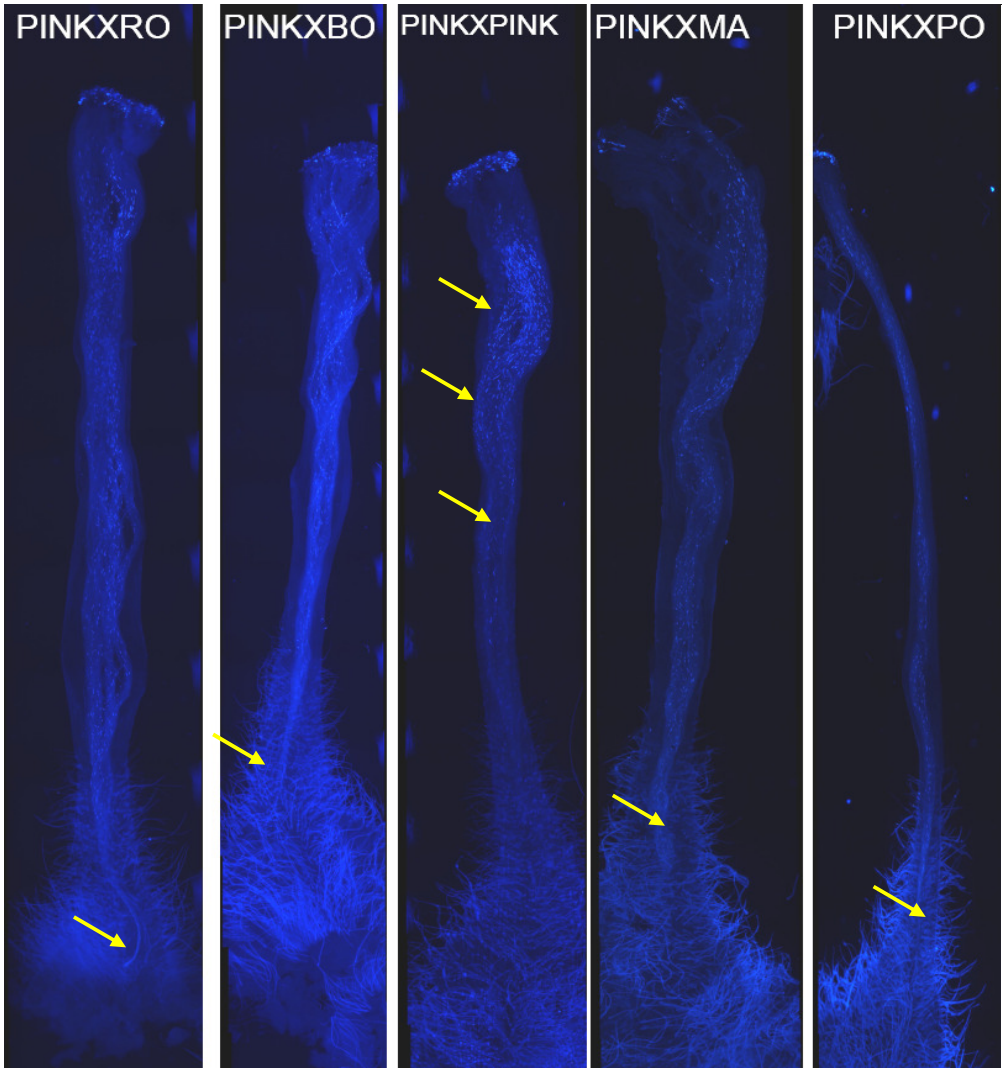


Figure 3.2c.

Illustrating the pollinated pistils of PINKCOT after 72 hours. As could be seen the pollen tubes with *S22* genotype of ROBADA (*S8S22*) have reached into the ovary. This photo clearly indicates the compatible reaction between pistil and pollen tubes in this combination. Thus we confirm the cross compatibility among PINKCOT as female an ROBADA, BORA(*ScS9*), MAYA(*S9/S2*) and PORTICI (*S2S17′*). The pollen tubes are visible clearly for the callus deposition along it′s growth down toward ovary. Plenty of tubes could be seen at the first part of transmitting area, but finally a few of them are reaching to the basal point. Considering the *S8S9* genotype of PINKCOT, the arrested pollen tubes at the first half part of pistil length in PINKCOT × PINKCOT crossing is considerable, which demonstrates the self-incompatibility in this cultivar. The *S8* pollen tubes from ROBADA (*S8S22*), is arrested and the remains should be *S22* which have continuous growth. All *Sc* and *S9* pollens in the crossing PINKCOT × BORA, are able to grow and *S9*, *S2* from MAYA and *S2, S17′* from PORTICI will grow, too. (RO=ROBADA, BO=BORA, PINK=PINKCOT, MA=MAIA, PO=PORTICI).

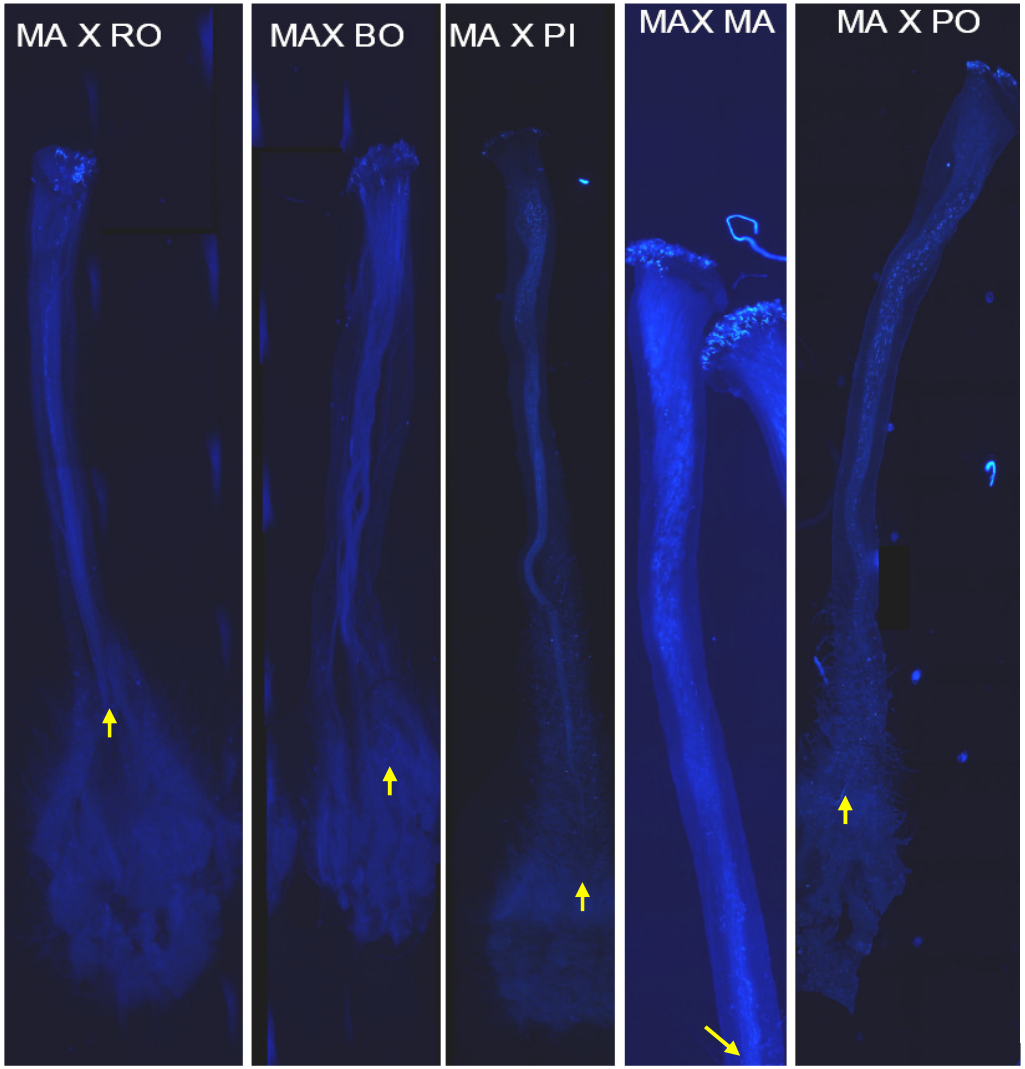
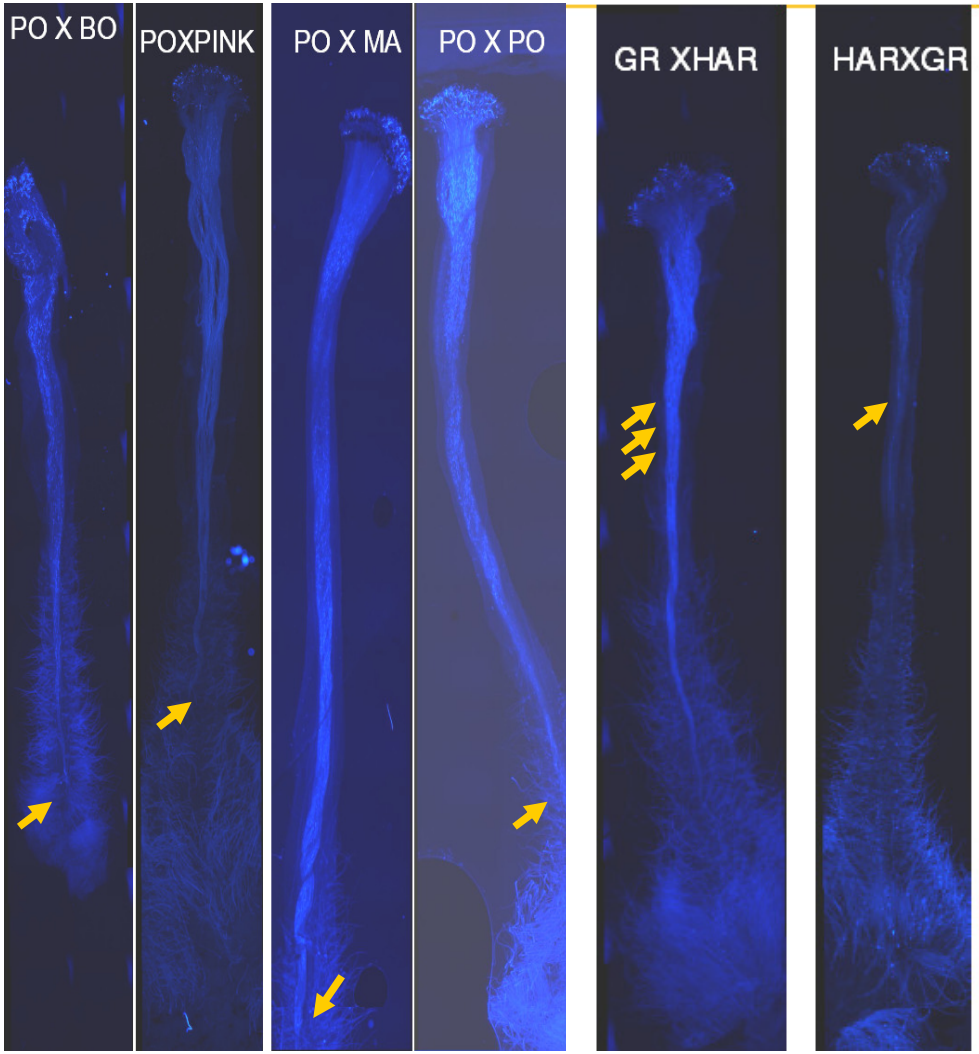


Figure 3.2d.

MAYA (*S9S2*) is self-compatible as the self pollen tubes could be followed till the basal point. It is considerable to study deeply the situation of *S2* later which has inherited from PORTICI as one of MAYA′s parents. Both MAYA and PORTICI are self-compatible in field studies. Moreover the pollens from other five cultivars used here were allowed to growth and reach to the basal point or ovary. The good pollen germination on stigma is considerable (RO=ROBADA, BO=BORA, PINK=PINKCOT, MA=MAIA, PO=PORTICI.



Figurer 3.2e.

The micrographic photos of pollinated pistils of PORTICI (*S2S17´*) and for reciprocal crosses: HARGRAND (*S1S2)* × GOLDRICH (*S1S2)* and GOLDRICH × HARGARND which are shown as reference for negative control for self-compatibility. The photos are illustrating the whole view of pistils from stigma to ovary, to facilitate following the pollen tube growth(RO=ROBADA, BO=BORA, PINK=PINKCOT, MA=MAIA, PO=PORTICI, GR=GOLDRICH and HAR=HARGRAND). Actually, the incompatible pollen tubes are arrested before passing half of their pathway toward ovary. Some factors such as the age of flowers has side affect on the possibility of pollen tubes growth, very young (immature) flowers or very old ones might have different behavior for the trait of self-incompatibility which is related to presence or absence of biochemical barriers. The pistil of PORTICI (*S2S17´*) is interacting with pollens: *S2, S17´,Sc, S8, S9* among the above crosses. The pollen tubes of *S2* and *S17´* had to be rejected which is true for *S2*, but for the *S17´*, interestingly in the case of PORTICI self crossing, the pollen tubes were observed in the ovary. The PORTICI has phenotypes as self-compatible in field, too.

3.1.3. genetic variability in locus S among studied cultivars (SSR markers)- In order to preliminary evaluation of genetic variability surrounding the S-locus , two SSRs closely linked to this trait were analyzed (Table 3.6). Of course two SSRs are not enough to demonstrate the variability among the varieties but in this case they are employed just for preliminary evaluation.

Table 3.6 - SSR markers series used to verify the amplification efficiency (Dondini, 2007)

Species	SSR series	Motif	Author
Peach 2002	CPPCT	CT,GA,TC	Aranzana et al.
Peach 1999	UDP010	AC,AG,CA,GA,TG	Cipriani et al.

Polymorphic alleles were scored as present or absent (0/1). Band scoring was analyzed using NTSYS 2.0 analysis software. Genetic relationships among genotypes were studied by UPGMA (Unweighted Pair Group Method with Arithmetic averages) cluster analysis (Table 3.7). Referring to figure 3.3, and the results of S-genotyping presented later in this chapter, it could be found that the varieties ALTERA and CORNIA, are estimated to be more similar and PIEVE, PIEVE TARDIVA and BO92639095, too. Interestingly two main groups could be distinguished according to calculated dedrogram. In one group all varieties containing the Sc and S1 alleles and on the other group none of these alleles were identified during genotyping. In some cases the results do not seem to correspond the S-genotyping results which indicating the non-applicability of the SSR markers to analyze the S-locus. For example the NINFA did not show polymorphism respect to BORA and PORTICI in this region.

Table 3.7 - SSR Matrix obtained by two SSRs located in locus S

No.	**Var.**	**CT1**				**UDP010**									
	Identified Bands(Fragments):	A1	A2	A3	A4	B1	B2	B3	B4	B5	B6	B7	B8	B9	B10
1	ALTERA	1	0	0	1	1	1	0	0	0	0	0	0	0	0
2	BO92639095	1	0	0	1	1	1	0	0	0	0	0	0	0	0
3	BO93622312	1	0	0	1	1	1	0	0	0	0	0	0	0	0
4	BORA	1	0	0	0	0	0	1	1	0	0	0	0	0	0
5	CORNIA	1	0	0	1	1	1	0	0	0	0	0	0	0	0
6	HARCOT	1	0	0	1	1	0	0	0	1	0	0	0	0	0
7	GHEYSI	1	0	0	1	1	0	0	0	0	0	1	0	0	0
8	NADERI	1	1	0	0	0	0	0	0	0	1	0	0	0	0
9	KYOTO	1	0	0	0	0	1	0	0	0	0	0	0	0	0
10	LILLYCOT	1	0	1	0	0	0	0	1	0	0	1	0	0	0
11	MAIA	1	0	0	0	0	0	0	1	0	0	0	1	0	0
12	NINFA	1	0	0	0	0	0	1	1	0	0	0	0	0	0
13	PETRA	1	0	0	1	1	1	0	0	0	0	0	0	0	0
14	PIEVE	1	0	0	1	1	1	0	0	0	0	0	0	0	0
15	PIEVE TARDIVA	1	0	0	1	0	1	0	0	0	0	0	0	1	0
16	PINKOT	1	0	0	0	0	0	0	1	0	0	0	0	0	0
17	PORTICI	1	0	0	1	0	1	0	0	0	0	0	1	0	0
18	REALE	1	0	0	1	0	0	0	0	1	0	0	1	0	0
19	ROBADA	1	0	0	0	0	0	0	1	0	0	0	0	0	1
20	SAN CASTRESE	1	0	0	1	0	1	0	0	1	0	0	0	0	0
21	TARDIF DE VALENCE	1	0	0	1	0	1	0	0	0	0	0	0	0	1
22	YAMAGATA 3	0	0	0	1	1	1	0	0	0	0	0	0	0	0
23	LITO	1	0	0	1	0	0	0	1	1	0	0	0	0	0
	No. Of alleles	**22**	**1**	**1**	**15**	**9**	**12**	**2**	**7**	**4**	**1**	**2**	**3**	**1**	**2**

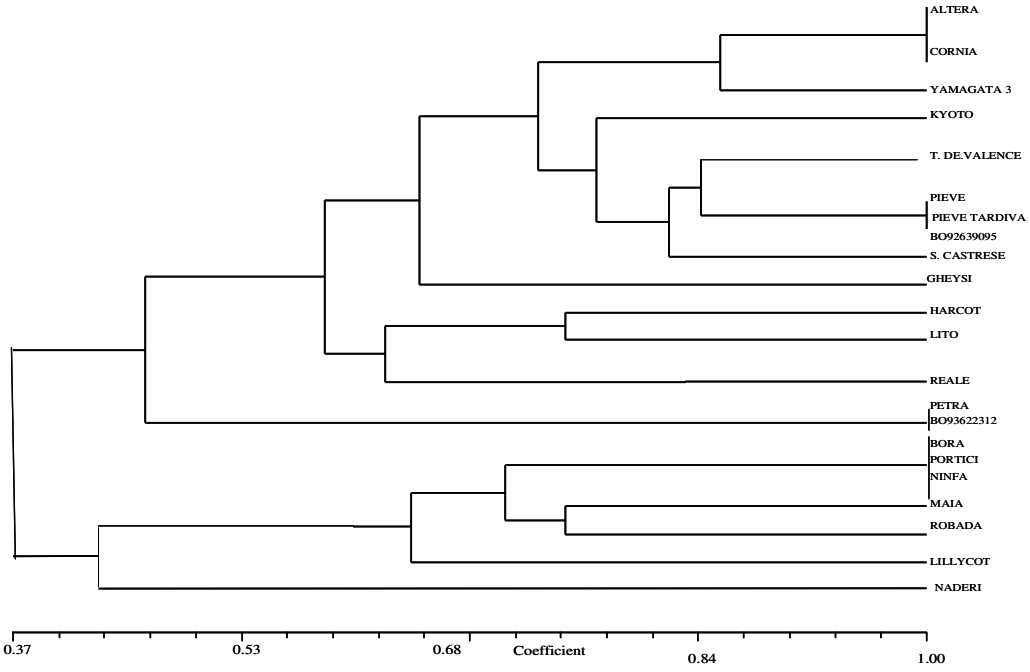


Figure 3.3 -Dendrogram obtained by UPGMA cluster analysis based on the mean character difference distances among apricot cultivars based on two SSR markers applied to verify polymorphism in locus *S.*

3.1.4. Identification and characterization of *S-RNases*

The primers EM-PC2consFD, EM-PC3consRD, EM-PC5consRD, SRC, PaconsI, PaconsII, applied to amplify the fragments of 1st or 2nd introns, were successful, although their efficiency is variable. In the Table 3.8 their efficiency is illustrated according to the patterns obtained via electrophoresis on agarose gel 2%. We examined all these previously designed primers amplifying fragments of the *S-RN*ase haplotypes containing 1st or 2nd intron for genotyping our cultivars and all of them, more or less, were useful. Among them SRC produced very clear PCR patterns, which led to cloning of fragments in order to sequencing.

Table 3.8 - The primers applied to amplify the fragments of 1st or 2nd intron (not strong but visible bands are considered).

No. Cultivar		Primers				
		Pacons I (F+R)	Pacons II (F+R)	EM-PC2consFD		SRC (F+R)
				EM-PC3consRD	EM-PC5consRD	
1	ALTERA	++	++	++	-	++
2	BO92639095	++	++	+	-	++
3	BO93622312	++	++	+	-	++
4	BORA	++	+	+	+	++
5	CORNIA	++	++	+	-	+
6	HARCOT	++	+	+	-	+
7	GHEISI	+	+	++	+	++
8	NADERI	++	+	++	+	++
9	KYOTO	+	+	+	+	++
10	LILLYCOT	+	+	++	+	++
11	MAIA	+	++	++	+	++
12	NINFA	++	++	+	+	++
13	PETRA	+	++	+	-	++
14	PIEVE	++	++	-	-	++
15	P. TARDIVA	++	++	-	-	++
16	PINKCOT	++	+	++	+	++
17	PORTICI	++	++	++	-	++
18	REALE	+	+	-	-	+
19	ROBADA	+	++	++	+	++?
20	S.CASTRESE	++	++	-	-	++
21	TARDIF DE VALENCE	++	++	-	-	++
22	YAMAGATA 3	+	+	+	-	++
23	LITO	++	+	+	-	+
24	GOLDRICH	++	++	+	+	++
Efficiency		40/48	38/48	27/48	10/48	44/48

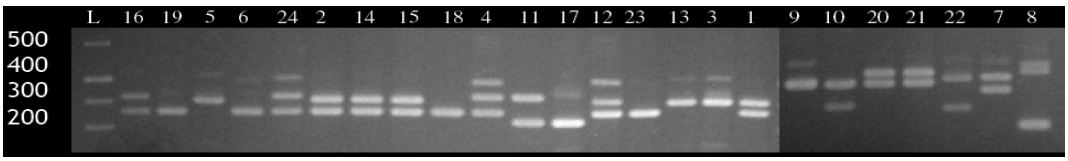


Figure 3.4 - The efficiency of primer SRC(F+R) in amplifying fragments of the *S-RN*ases 1st intron . 1-ALTERA, 2- BO92639095, 3- BO93622312, 4-BORA, 5-CORNIA, 6-HARCOT, 7-GHEYSI, 8-NADERI, 9-KIOTO(KYOTO), 10-LILLYCOT, 11-MAYA(MAIA), 12-NINFA, 13-PETRA, 14-PIEVE, 15-PIEVE TARDIVA, 16-PINKCOT, 17-PORTICI, 18-REALE DI IMOLA, 19-ROBADA, 20-SANCASTRESE, 21-TARDIF DE VALENCE, 22-YAMAGATA3, 23-LITO and 24-GOLDRICH.

Primer EMPC: Three degenerate primers, flanking the second intron, which have been designed based on the second, third and fifth rosaceous *S-RN*ase conserved regions (Ushijima et al. 1998) namely; EM-PC2consFD, EM-PC3consRD and EM-PC5consRD, also were used in C2 + C3 and C2 + C5 combinations. In our case these set of primers were able to genotype all of the varieties except REALE D'IMOLA, and NADERI, which alleles were not easy to amplify. In the case of REALE DI IMOLA, which it's S-genotype is proposed ScSc, it becomes clear considering the applicability of this marker in amplifying heavy alleles.

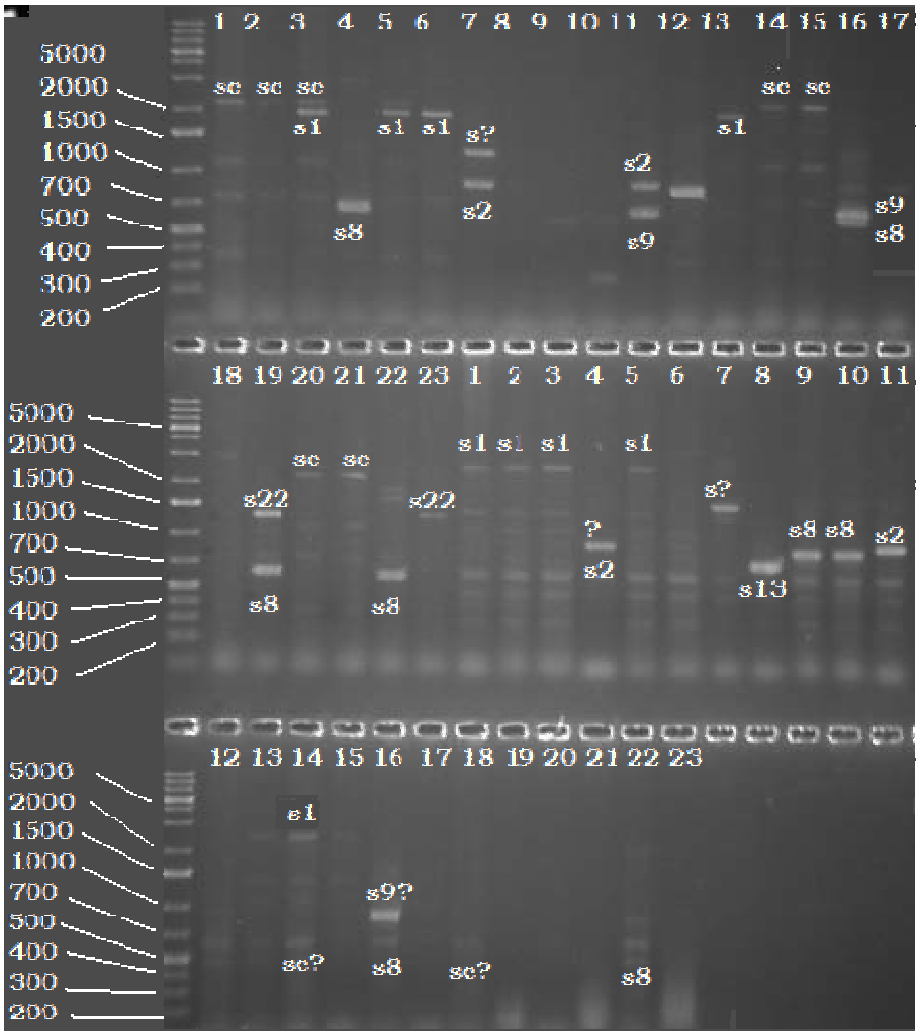


Figure 3.5a. The pattern obtained by EMPC primers: First series 1 to 23 obtained by EMPC2 (F)+EMPC3 (R) combination and the second series are obtained by EMPC2(F)+EMPC5 (R) combination . 1-ALTERA, 2- BO92639095, 3- BO93622312, 4-BORA, 5-CORNIA, 6-HARCOT, 7-GHEYSI, 8-NADERI, 9-KIOTO(KYOTO), 10-LILLYCOT, 11-MAYA(MAIA), 12-NINFA, 13-PETRA, 14-PIEVE, 15-PIEVE TARDIVA, 16-PINKCOT, 17-PORTICI, 18-REALE DI IMOLA, 19-ROBADA, 20-SANCASTRESE, 21-TARDIF DE VALENCE, 22-YAMAGATA3, 23-LITO and 24-GOLDRICH. the S-allele is assigned for each band according to references.

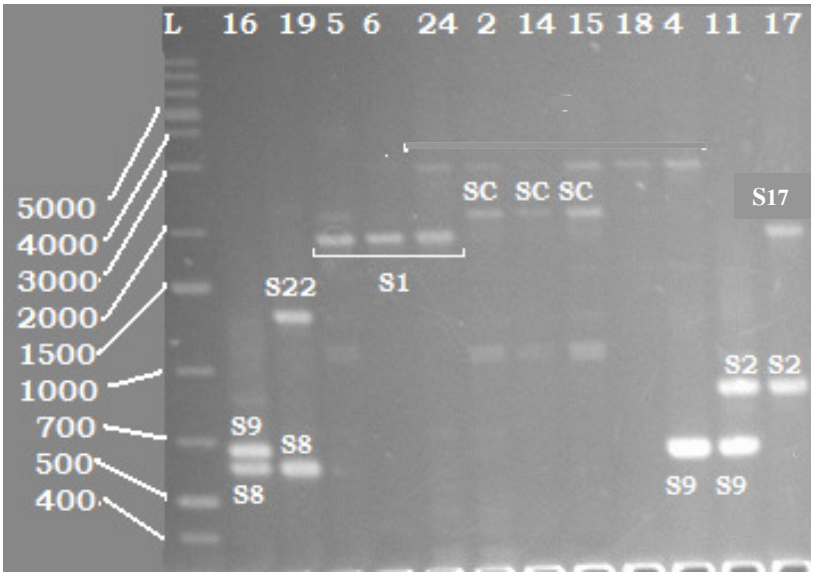


Figure 3.5b. The pattern obtained by EMPC2 (F)+EMPC3 (R) combination. 2- BO92639095, 4-BORA, 5-CORNIA, 6-HARCOT, 11-MAYA(MAIA), 14-PIEVE, 15-PIEVE TARDIVA, 16-PINKCOT, 17-PORTICI, 18-REALE DI IMOLA, 19-ROBADA and 24-GOLDRICH.

EM-PC2consFD and EM-PC3consRD have been designed for the amplification of the second intron region of sweet cherry according to Sutherland et al. In (2004), but using them was successful to amplify fragments of both introns in our case, apricot s-alleles, which is in agreement with Halasz, 2005 foundings. The applied consensus primers amplified 1 or 2 bands per accession in 24 apricot cultivars (Figure 3.5a).

In the figure 3.5b the results about S-genotyping of BORA, MAYA and PORTCI are highlighted. It is very clear that MAYA received from its mother PORTICI the allele S2 while from the father BORA the allele S9 instead of Sc. This result opens a new question concerning the source of self-compatibility of PORTICI and MAYA which are not carrying the Sc allele. This crucial point has to be more deeply investigated from molecular point of view in the next future, but this result is in agreement with Villanova et al., 2006 that described the Cultivar CANINO as carrier of a mutated S2 allele which confers self-compatibility.

Primer Pacons: Primer sets Pacons I(F+R) and Pacons II(F+R) used for amplifying 1st intron and 2nd intron respectively.

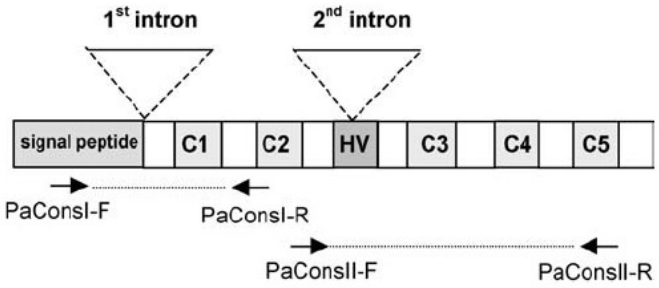


Figure 3.6 -Structure of a *Prunus S-RNase* with intron locations and position of consensus primers (not to scale) (Sonneveld et al., 2003).

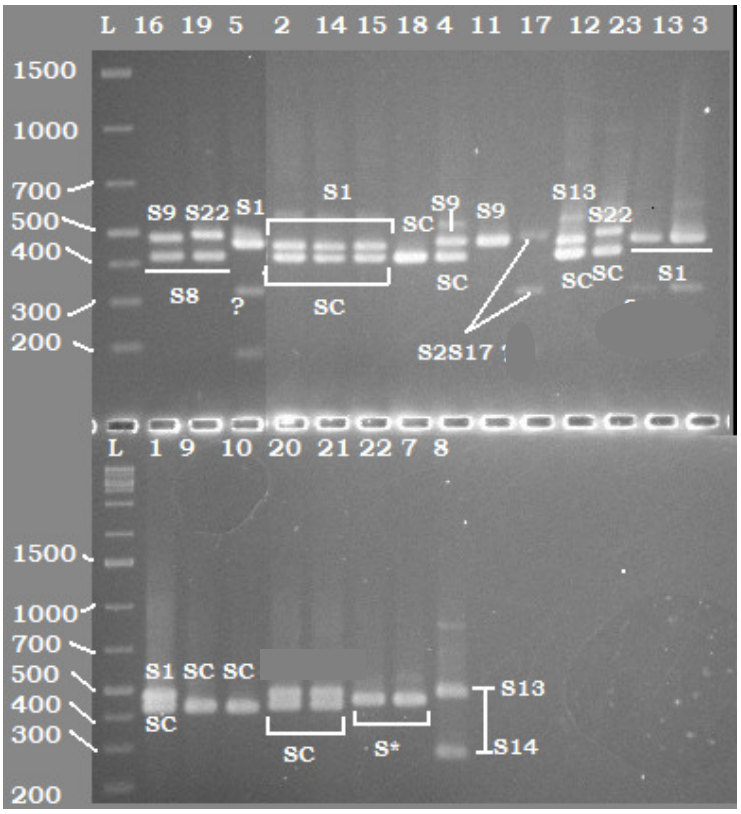


Figure 3.7. Pacons I amplifying 1st intron of *S-RN*ase gene. 1-ALTERA, 2- BO92639095, 3-BO93622312, 4-BORA, 5-CORNIA, 6-HARCOT, 7-GHEYSI, 8-NADERI, 9-KIOTO(KYOTO), 10-LILLYCOT, 11-MAYA(MAIA), 12-NINFA, 13-PETRA, 14-PIEVE, 15-PIEVE TARDIVA, 16-PINKCOT, 17-PORTICI, 18-REALE DI IMOLA, 19-ROBADA, 20-SANCASTRESE, 21-TARDIF DE VALENCE, 22-YAMAGATA3, 23-LITO and 24-GOLDRICH.

It could be found that the primer sets: Pacons I and II are generating fragments within the range of 200-500bp and 400-2700 bp, respectively. The Pacons I(F+R) sets helped to identify the alleles: S1, SC, S8, S9, S13, S14, S22 and two not known ones which are marked as S* or question mark. The other set PaconsII (F+R) identified the S1, S2, S8, S17′ and S22 but not Sc. Combining all patterns obtained by these primers (figures 3.7 and 3.8) it was possible to confirm the results obtained by EMPC primer sets.

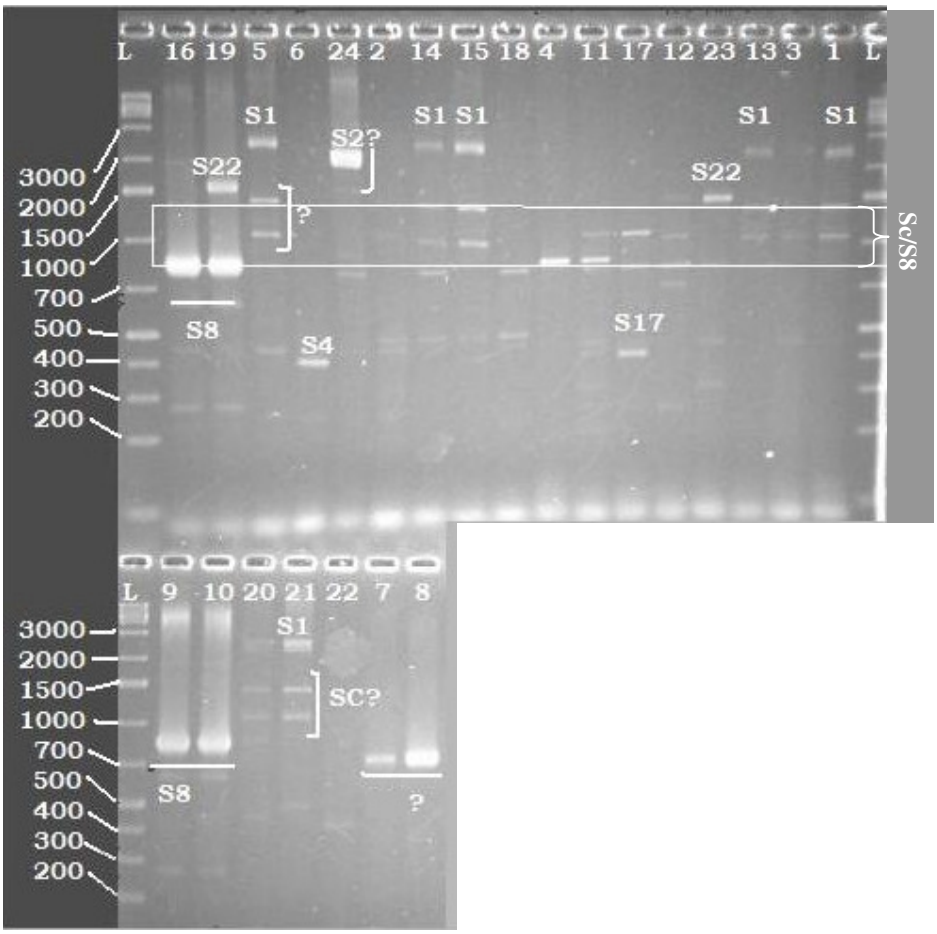


Figure 3.8. Pacons II amplifying 2nd intron of *S-RN*ase gene and the S-allele numbers assigned to each band. 1-ALTERA, 2- BO92639095, 3- BO93622312, 4-BORA, 5-CORNIA, 6-HARCOT, 7-GHEYSI, 8-NADERI, 9-KIOTO(KYOTO), 10-LILLYCOT, 11-MAYA(MAIA), 12-NINFA, 13-PETRA, 14-PIEVE, 15-PIEVE TARDIVA, 16-PINKCOT, 17-PORTICI, 18-REALE DI IMOLA, 19-ROBADA, 20-SANCASTRESE, 21-TARDIF DE VALENCE, 22-YAMAGATA3, 23-LITO and 24-GOLDRICH. It seems that within the range of approximately 800bp to 1500bp the presence of Sc allele is expected.

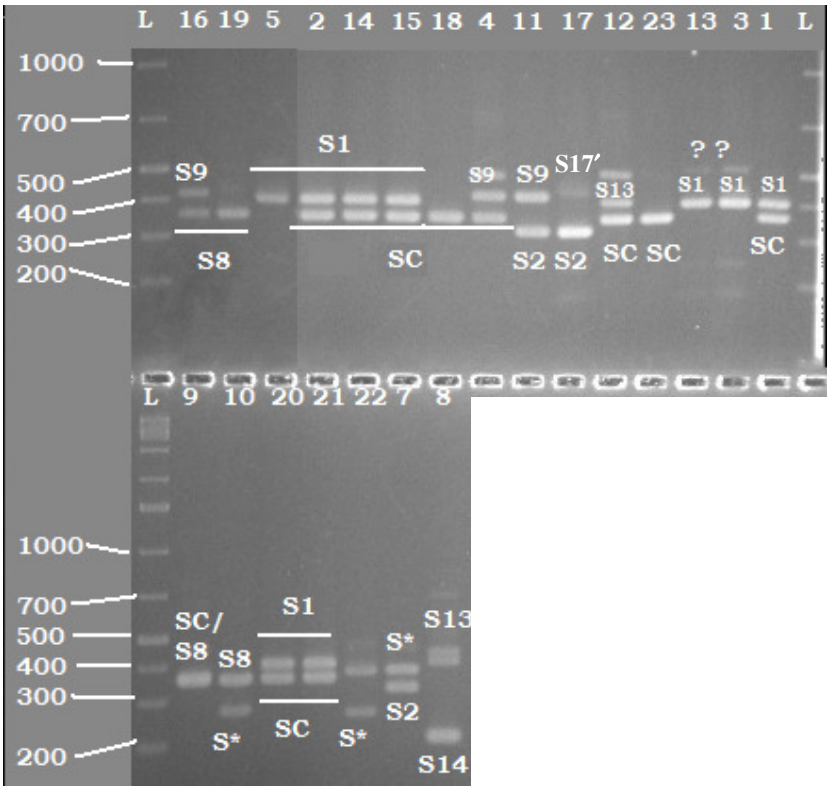


Figure 3.9. Electrophoresis pattern obtained using SRC(F+R) primer and the assigned S allele for each amplified fragment; 1-ALTERA, 2- BO92639095, 3- BO93622312, 4-BORA, 5-CORNIA, 6-HARCOT, 7-GHEYSI, 8-NADERI, 9-KIOTO(KYOTO), 10-LILLYCOT, 11-MAYA(MAIA), 12-NINFA, 13-PETRA, 14-PIEVE, 15-PIEVE TARDIVA, 16-PINKCOT, 17-PORTICI, 18-REALE DI IMOLA, 19-ROBADA, 20-SANCASTRESE, 21-TARDIF DE VALENCE, 22-YAMAGATA3, 23-LITO and 24-GOLDRICH.

According to the results obtained by using different sets of primers, as the primer pairs SRC could amplify the first intron of *S-RN*ase, and has been applied previously by researchers the results show that this genomic region is less variable in size (from 120 bp to 330 bp and thus less specific than 2nd intron which vary in size from 150 to 2700 bp. Referring to reported size of previously detected S-alleles (Vilanova, 2005, Halasz, 2007) it seems that the size of 1st intron has not exceeded this range yet. Combining the legible patterns obtained by PCR-product electrophoresis on agarose gel and sequence analysis enabled the S-genotyping of accessions evaluated in this study.

Although, a few questions are present yet which is due to lack of colony creation for corresponding allele. To cover this case in the second round we employed the PCR product using EMPC primers with C2 (as Forward) + C3 (as Reverse) combination to amplify fragments containing 2nd intron. The products obtained in this way were cloned according to the procedure previously explained in chapter 2. This procedure

was successful for NADERI, LILLYCOT, NINFA, PINKCOT, ROBADA and YAMAGATA3 and led to sequence some remained allelic variants and confirmed the results obtained by SRC primer pairs.

3.1.5. Database comparison and alignments- PCR products were cloned and sequenced. The obtained *S-RNase* intron DNA sequences were compared for homology with already available and published sequences in the EMBL database using the FASTA algorithm. According to the high similarity (%), the specific allele denomination was assigned to each variant and in the case of very low similarity the variant was considered as a new. The phylogenic tree obtained based on homology in nucleic acid sequences for all *S-RN*ases identified up to date are presented in figure 3.10.

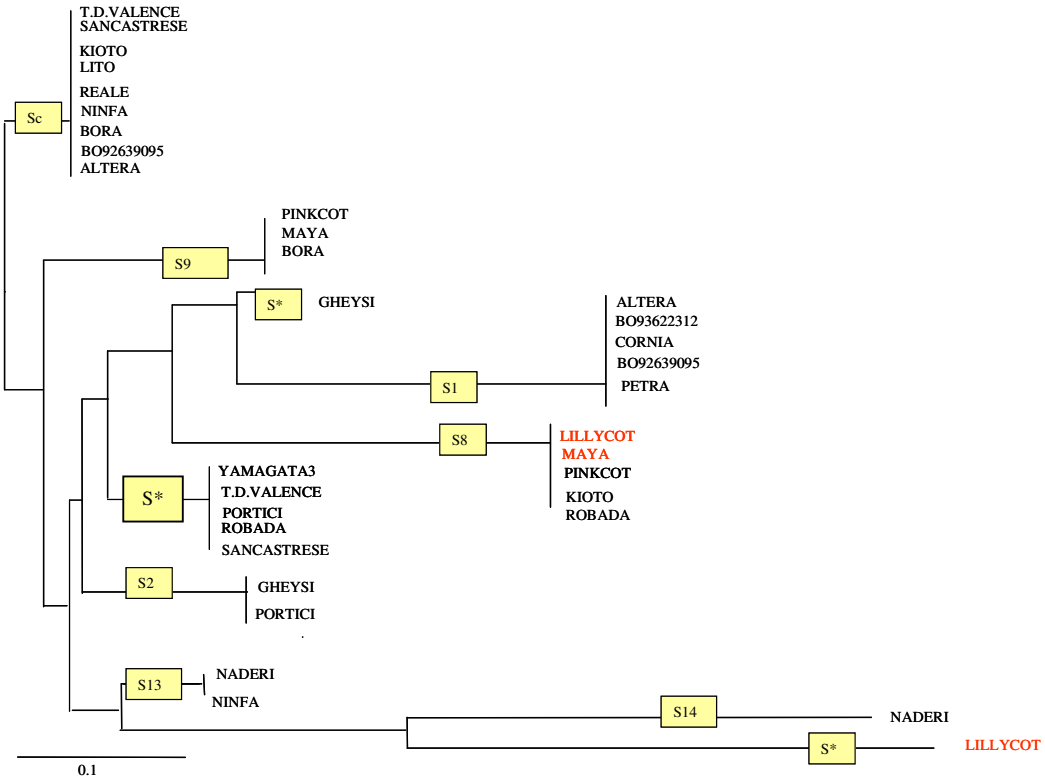


Figure 3.10. The Tree calculated from multiple alignments obtained according to sequences obtained for *S-RNase* in this work.

3.1.6. *S*-genotyping based on identified *S-RNases* – To identify the S-genotype of accessions the patterns obtained applying different previously mentioned primer combinations were studied and adjusted by eye. The size of the fragments appeared on gel (agarose and Polyacrylamide), were verified on the basis of the obtained sequence. determined/estimated referring to the markers applied. On the Table below the results are demonstrated.

Table 3.9-The varieties and the two band sizes obtained by sequence analyses for *S-RNase* 1st intron

No.	Var.	Size (bp)	Size (bp)	No.	Var.	Size (bp)	Size (bp)
1	ALTERA	353	400	13	PETRA	180	400
2	BO92639095	353	400	14	PIEVE	353	400
3	BO93622312	-	400	15	PIEVE TARDIVA	353	400
4	BORA	353	-	16	PINKCOT	327	-
5	CORNIA	-	400	17	PORTICI	328	-
6	HARCOT			18	REALE	353	353
7	GHEISI	325	376a	19	ROBADA	-	419
8	NADERI	202	400	20	SAN CASTRESE	353	419
9	KYOTO	353	-	21	TARDIF DE VALENCE	353	419
10	LILLYCOT	353	267b	22	YAMAGATA 3	-	419
11	MAIA	354	328	23	LITO	353	-
12	NINFA	353	400	24	GOLDRICH		

The primer SRC is amplifying fragments of 1st intron , as could be seen the variability in size for this intron is not enough to suffice for assigning S number. The varieties: ALTERA, BO92639095, BORA, KYOTO, LILLYCOT, NINFA, PIEVE, PIEVE TARDIVA and LITO all are sharing the same sized band; 353 bp, which is corresponding to Sc 1st intron according to already published ones. The other most common band sized 400 bp, is specific for S1 allele which could be assigned to CORNIA, ALTERA, BO92639095, BO93622312, PETRA, CORNIA, PIEVE, and PIEVE TARDIVA. We found the same band size for other allelic variants which gives doubtful understanding about NADERI, NINFA for allele S13. the marked S alleles with strikes indicates the probable new alleles as no correspondents were found in literatures to assign specific allele number to them. The determined variability

according to use of this primer is low, but looking from positive view, this primer set is capable of indicating the self-compatibility allele; Sc. The allele that has a size of 419 bp which was found in YAMAGATA3, T. DE VALENCE, ROBADA, PORTICI and SANCASTRESE represents an heterogenic group of alleles that have high homology in these region, in fact F-box sequencing evidenced that ROBADA is carrying the S22 allele, PORTICI the S17′ (highly homologous with S17). To clarify this point better, longer sequences of this allele are needed.

Applying other designed primer sets specific for 1st intron of *Prunus* species that is EMPC(2F+3R) for some of varieties were useful to clarify part of doubtful results. For example the 266 bp sized band which is corresponding to S8 is common among two varieties: LILLYCOT and ROBADA as could be confirmed referring to sequence alignment analysis.

Table 3.10- PCR products sizes using EMPC2+3 primer

No.	Variety	Size(bp)	No.	Variety	Size(bp)
8	NADERI	491	16	PINKCOT	556
10	LILLYCOT	266	19	ROBADA	266
11	MAIA	583	22	YAMAGATA3	337
12	NINFA	592			

This primer has been worked very well to identify the alleles : Sc, S1 to S6 in the previous research by Sutherland et al., 2004. For the NADERI, LILLYCOT, MAIA, NINFA, PINKCOT, ROBADA and YAMAGATA3 which this primer was applied, the band size obtained after sequencing analysis (Table 3.10), are out of the range examined by them, thus non of these varieties owning these S alleles. In the case of LILLYCOT and ROBADA, it could be confirmed by sequence analyses which is homologous to S8.

3.1.7. Identification and characterization of *F-Boxes*

In order to identify F-box proteins two sets of primers, *SFB*c(F+R) and *SFB*(F+R) were used. The primers; *SFB* and *SFB*c-R; which have designed according to the alignment of European apricot *SFB*s S1(AY587563), S2(AY587562) and S4(AY587565) (Romero et al. 2004), were able to identify eight F-box allelic variants, then it is concluded these primers sets could be used for F-box typing in apricot as a supplementary part beside the *S-RN*ase based S-genotyping. The amplification of the fragments in F-box was all right but the amplified fragments were not distinguishable on agarose gel because of the low variability of the amplified fragments. The later sequence analysis indicated that the problem is arising from the high degree of identity in the allelic variants of F-box proteins The size of fragments are approximately 694bp for all tested varieties. Then the Single Strand Conformation Polymorphism method was applied (the details were described in chapter 2). This technique helped to identify *SFB* variants among the studied cultivars before sequencing.

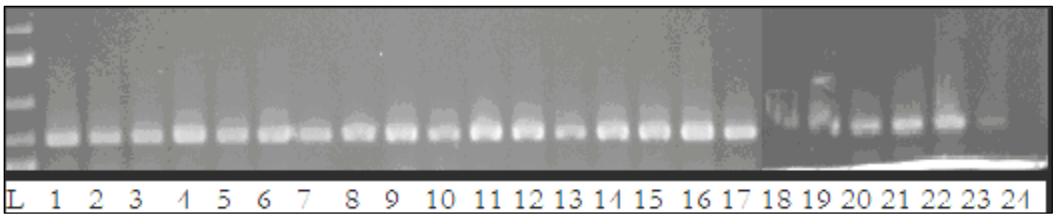


Figure 3.11. The identical bands of *SFB* fragments appeared on agarose gel. Although the desired fragments were amplified, but they were not separated by agarose gel (2%) electrophpresis. The 1kb ladder was used. The samples were left to 1.5-2 hours to run at 100V.

the PCR-product of samples were prepared using the same protocol and program for PCR and the procedure previously described in details in part 2.4.8 in chapter 2.

In the figure 3.12 the results of possibility to discriminate the amplified fragments of SFBs during colony-PCR are demonstrated. According to hopeful results of amplification, we isolated all possible colonies to extract DNA necessary for sequencing.

Thirty six discriminated fragments corresponding to specific colonies were used to sequence analyses.

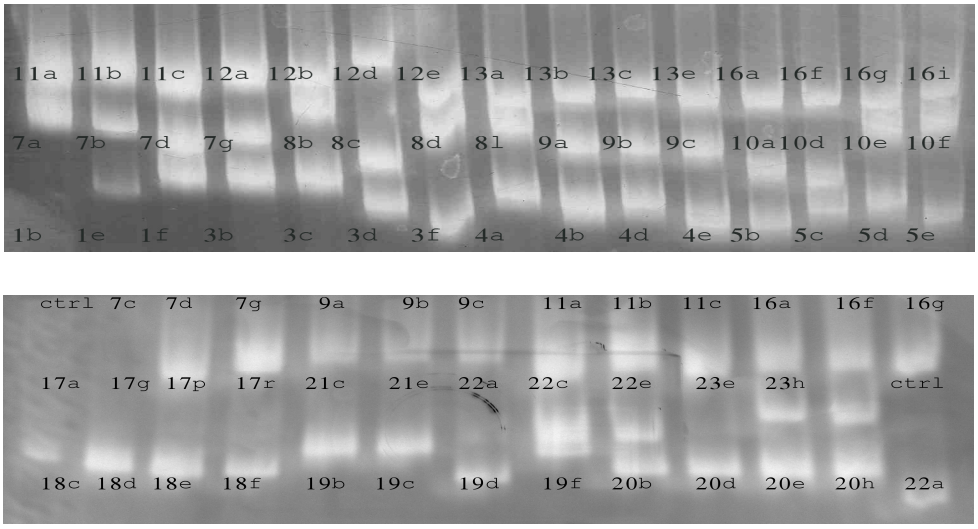


Figure 3.12. The discrimination of allelic variants of *SFB* after colony-PCR via SSCP Technique on MDE gel running overnight. As could be seen, 34 colonies were isolated for transformation procedure. They are corresponding to ten *SFB* sequences which already have been submitted to EMBL with high identity.

3.1.8. S-genotyping based on identified *SFBs* – To assign specific S to each amplified fragment the patterns obtained on agaros gel were not useful. The electrophoresis on Polyacrylamide gel gave doubtful results. In this case the sequence analyses had more clear results which the fragment colonies obtained by SSCP technique were used as DNA source in sequencing procedure. Comparing the results with those already submitted to EMBL helped to assign the right genotype for each accession. In the Figure 3.13 are illustrated the results obtained by SFB sequencing that confirm the allelic variants of accessions and clarified the results based on S-RNase genotypings.

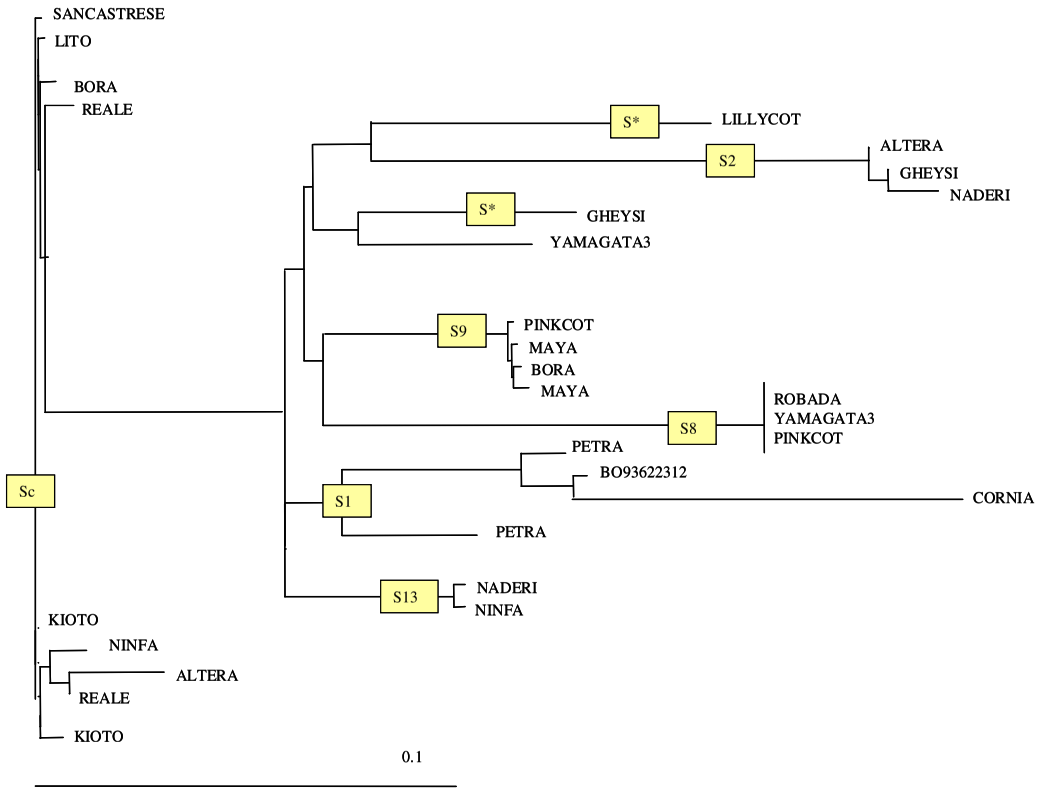


Figure 3.13-The rectangular clandogram view of homology amoung the identified sequences of SFB alleles in this work.

3.2. Results integration and discussion:

3.2.1. S-alleles segregation among studied varieties: The right situation of the S-genotype for each apricot variety is discussed below. Combining all results obtained by approaches applied in this work, the diploid *S* combination could be assigned for each variety. Investigations to determine the S genotype of individuals through length polymorphism of introns associated with the stylar *S-RN*ase gene and the pollen *SFB* gene reveals both new and already published *S* intron profiles which the new ones do not correspond to those of known S alleles in data bank.

- ALTERA (S1Sc): Referring to the literature this variety is the progeny of (HARCOT(S1S4) × (SAN CASTRESE, *ScS-* × REALE DI IMOLA, *ScSc*)), it was expected that this variety to show compatibility trait in our tests. The tree was not available for field tests then only DNA was applied to research on it. The primers applied were able to predict the presence of allele *S1*. The other allele is Sc, derived from compatible parents in it′s pedigree.

- BO92639095 (S1Sc): Due to the same pedigree of this accession as PIEVE and PIEVE TARDIVA; (HARCOT(*S1S4*) × REALE DI IMOLA), the same S-genotype for it detected. The fruit set determined in field for this accession was 10%, enough to phenotype it as self-compatible. The next step of molecular analyses confirms the results obtained for PIEVE and P.TARDIVA. Thus the S1 is derived from HARCOT and the Sc is from another partner.

- BO93622312 (S1-): This accession is derived from PETRA selfing which itself is a progeny of GOLDRICH(S1S2) × PELESE DI GIOVANNIELLO (S?S?). The PCR-product analyses indicates the *S1* in it′s *S*-locus. The field results with 6.5% of fruit set although is not very trustable to give a stable conclusion for self-compatibility. It has the same PCR patterns of its progenitor PETRA and a second allele was not identified. This genotype as source of self-compatibility has to be, analogously to PETRA, further investigated

BORA (S9Sc) (EARLY BLUSH × PA7005-2): In literature BORA has considered to be partially self-compatible. All three approaches applied here are confirming its self-

compatibility. BORA set fruit 14% in hand pollination tests in field. Monitoring pollen tube growth experiments confirmed it′s self-compatibility within two interval years and our results obtained via PCR analyses referring to electrophoresis patterns shows the Sc allele presence. The sequencing and subsequently, alignment studies indicate the high homology with already submitted sequence in genebank (EMBL), under DQ422947 for *S-RNase* and DQ422946 for *SFB* part, both for allele *Sc*. For the second allele the agarose gel patterns and F-box sequencing indicated the presence of *S9.*

More over this genotype is in agreement with MAYA *(S2S9)* as progeny of PORTICI × BORA, in which the presence of the allele *S9* is also demonstrated.

- CORNIA (S1S?): This varity is from NJA1 × BELLA DI IMOLA crossing. We have not field data for this variety. Presence of S1 was confirmed by experiments of both, *S-RNase* and *SFB* homology. The second allele was not detected but it′s not Sc.

- HARCOT (As reference; S1S4 according to literatures; Burgos et al., 2004)

- GHEYSI (S2S*): An Iranian cultivar with unknown pedigree, was considered to be self-incompatible (personal communication), demonstrated two amplified fragments during PCR-product electrophoresis one which could propose to be new S-allele (S*). Sequence analyses shows the S2 allele based on *S-RNase* and *SFB* homology. For the second allele there is no corresponding sequence in EMBL database with high homology.

- NADERI (S13S14): Also this cultivar has described as self-incompatible to our knowledge. The similar analyses proves the lack of Sc allele in its genome. The two alleles are homologous with *S13* and *S14*. The band of S13 is corresponding to the same allele in NINFA.

- KYOTO (SCS8): With unknown pedigree, demonstrates full self-compatible phenotype in field having 42.5% frit set. In electrophoresis patterns always show one amplified fragment might be due to the correspondence of Sc and S8 with many

primer pairs specific for S-RNases. Its S-genotype was confirmed after F-box sequencing.

- LILLYCOT (S8S**): This cultivar is reported as self compatible (unofficial report by CRPV, Research Agency of Emilia Romagna Region, 2009). We could not verify this report in field analyses and we can just comment the data obtained from PCR based experiments and from S-RNase and F-box sequencing. We could indicate the presence S8 instesd of Sc in its S-genotype. the other allele did not correspond to any other known S-allele. Therefore we consider this finding as a new allele indication (S**).

- MAIA (S2S9): This cultivar is a result of the crossing between PORTICI (female) and BORA (male) which both are self-compatible cultivars. Thus the MAIA might be a self-compatible cultivar (75% probability, if they share only one Sc allele). The field pollination confirms this propose and the pollen tube growth was followed and monitored reaching the basal point and ovary during controlled condition pollinating. Its proposed S-genotype is confirmed by the results collected in its parents PORTICI and BORA. The PCR patterns clearly indicated that MAIA did not receive the Sc allele from BORA and its self-compatibility has to derive from the S2 of PORTICI perhaps mutated or in the RNase or in the F-box, analogously to what described by Villanova et al., 2006. These aspects have to be better clarified in longer sequences of these genes.

- NINFA (ScS13): Concerning this cultivar we found two different reports for it′s compatibility trait. NINFA is reported as a self-incompatible cultivar (Halasz, 2007), and also it known as a self-compatible in Italy and Spain. The tree was not available in field to evaluate the self-compatibility via hand pollination for this cultivar. PCR-products and related sequencing indicated the presence of both S13 and Sc instead of S1, and S2 reported by Halasz, 2007. As further confirmation of our interpretation it has to be considered the pedigree of NINFA that is OUARDI × TIRYNTHOS. The TIRYNTHOS is self-compatible European cultivar (Romero et al., 2003) and it is father of LITO that is carrying the Sc allele.

- PETRA (S1S-): Obtained from the crossing: GOLDICH *(S1S2)* ×PELESE DI GIOVANNIELLO. Field experiments confirms the self-compatibility by 8% fruit set indication. The PCR analyses shows the presence of one of two alleles. Sequencing confirms to be S1 both S1-RNase and SFB-1. For this genotype we have to confirm what have before reported for BO93622312.

- PIEVE (S1Sc): This variety is obtained by the crossing: HARCOT(*S1S4*) × REALE DI IMOLA. The phenotyping results show self-compatible behavior of this variety as the fruit set was determined 10%. The PCR analyses confirms the presence of *Sc* allele in the patterns obtained by electrophoresis. The cloning and subsequently the sequencing was not applied for this variety as along the PIEVE TARDIVA, both have the same parents and the same PCR products. Therefore these analyses are carried out for the third member of these parents progeny; BO92639095, and the results are generalized for these three members (S1Sc). Thus it could be found that the self-compatibility of this varieties are due to the REALE DI IMOLA which in literature it has phenotyped as self-compatible cultivar. Our sequence and alignment analyses proved the homozygous status *(ScSc)* of this cultivar, which indicates it′s high value as a parent in hybridization programs.

- PIEVE TARDIVA (S1Sc): The results are the same as PIEVE.

- PINKCOT (S8S9): With unknown pedigree, has been considered as self-incompatible cultivar which was approved via our field experiments. Two amplicons are appeared on gel with ~360bp and ~400bp sizes for first intron obtained by SRC (F+R) primer. SFB Sequence analyses indicate that they are S8 and S9.

- PORTICI (S2S17′): The field test pollination showed the self-compatibility of this cultivar and thus it is confirmed strongly. The fruit set records were 12.2%, 20% and 14.5%. The pollen tubes also were followed arriving at the basal point of style and into the ovary after 72 hours in hand pollinating experiments. The PCR analyses reveals the presence of two visible bands which led to clone them. One of the alleles is S2 and the other one has high homology with S17 which was called

S17′. S2 is in common with MAYA and in this case , self-compatibility of this variety is not derived from presence of Sc. Thus another source of self compatibility has to be better characterized as proposed by Villanova et al. 2006.

- REALE DI IMOLA (*ScSc*): No data is presented for this cultivar for its right situation about S-genotype. Our molecular-based experiments, reveals the presence of Sc allele as the sequence of amplified fragments is homologous to already submitted sequence under DQ422947 in EMBL for Sc-RNase part and this data was confirmed by sequencing it′s F-box. PCR patterns and sequencing are indicating that REALE DI IMOLA is homozygote *ScSc*. In all patterns obtained by gel electrophoresis one strong band appeared corresponding to *Sc*.

- ROBADA (S8S22): (ORANGE RED × K113-40). The field pollination tests are confirming the self-incompatibility of this cultivar, with fruit set; 0%, 0.8% and 2%. Following pollen tube tests also indicates the arresting of tubes at one-third of transmitting pathway to ovary. One pollen tube was observed at basal point which may be is due to an error. The amplified fragments produced by PCR were migrated distinguishable on agarose gel indicating two different alleles. The sequence analyses of S-RNase and F-box led to assign S8S22 a S-genotype for it.

- SAN CASTRESE (Sc-): 30% fruit set obtained via field controlled pollination and numerous reports are documenting strongly the self-compatibility of this cultivar. Thus we have evidence for Sc both by PCR and sequencing. The electrophoresis pattern on agarose gel indicated two strong and clear bands close to each other using PaCons primer sets and SRC. The partially sequence of the second allele obtained for 1st intron didn't match to any previously submitted ones to EMBL and it could be a new allele (best homology is with S1, 90%).

- TARDIF DE VALENCE (Sc-): The electrophoresis patterns and sequencing result always were the same as SAN CASTRESE for this cultivar. The field tests confirms it as self-compatible cultivar as 15% fruit set was determined after eight weeks from controlled pollination. For that it is confirmed what described for SANCASTRESE.

- YAMAGATA-3 (S8S-): All primers applied here were able to amplify the fragments belonging to 1st or 2nd intron of the *S-RN*ase gene in this cultivar. After sequencing the fragments the S8 allele was denominated but there is a second allele that didn't correspond to any EMBL sequences for apricot with high homology. The best homology (90.5%) was found with *SFB*27, the recently identified allelic variant for Chinese apricots. YAMAGATA 3 has been reported to show low self-compatibility according to Chung K. Et al. (2004), for that this allele has to be better characterized.

- LITO (scs22): (STARK EARLY ORANGE × TIRYNTHOS), Has been considered as self-compatible cultivar and here the molecular experiments confirmed the presence of allele Sc. The sequence analyses indicated the high homology of amplified DNA of 1st intron of *S-RN*ase and SFB with Sc sequence. The female parent of this cultivar is Stark Early Orange (S.E.O) which according to Alburquerque (2002), is self-incompatible but the father is TYRINTHOS which putatively is the donor of the Sc allele

- GOLDRICH (as reference; the self-incompatibility of this cultivar previously has been determined by controlled cross-pollinations by Egea and Burgos, 1996): This cultivar is well documented to have S1S2 genotype (Romero et al., 2004). Our field pollination experiments confirms it′s self-incompatibility.

Combining all together, accordingly, the results of S-genotyping is summarized in Table 3.12. For GHEYSI, LILLYCOT, YAMAGATA 3, SANCASTRESE and TARDIF DE VALENCE the new S-allele variations are proposed which needs to be confirmed later by extending the sequences to the whole genes.

Table 3.12- The S-genotype assigned for the varieties studied in this work.

	Variety	S-genotype		Variety	S-genotype
1	ALTERA	S1SC	13	PETRA	S1S-
2	BO92639095	S1SC	14	PIEVE	S1SC
3	BO93622312	S1S-	15	PIEVE TARDIVA	S1SC
4	BORA	SCS9	16	PINKOT	S8S9
5	CORNIA	S1S-	17	PORTICI	S2(S17`)
6	HARCOT	S1S4	18	REALE	SCSC
7	GHEYSI	S2Sa	19	ROBADA	S8S22
8	NADERI	S13S14	20	S.CASTRESE	SCS-
9	KIOTO	SCS8	21	TARDIF DE VALENCE	SCS-
10	LILLYCOT	S8Sb	22	YAMAGATA 3	S8S-
11	MAIA	S9S2/8	23	LITO	SCS22
12	NINFA	SCS13	24	GOLDRICH	S1S2

3.2.2. Applicability of the *SFB*-based *S*-genotyping procedure

The *S*-genotyping methods evaluated and used here to determine the *S* genotypes of cultivars of different origin will be useful for the breeding, selection and characterization of apricot cultivars.

This study confirms evidence that the *S-RNase* genes of the incompatible *S8* and compatible *SC* haplotypes are identical and that the analyses of F-box is important for right S-genotyping. Therefore, the *S-RN*ase PCR approach could not be enough to determine the compatibility phenotype. In contrast, the *SFB* consensus primers were able to identify SC phenotypes and further could distinguish between heterozygotes and homozygotes at the locus. Moreover, to distinguish alleles with similar size introns the RNase based genotyping gives doubtful results. In our experience it is better to perform the analyses by combining the results of several primers specific for *S-RNase-* and the *SFB* based PCR methods. In this way S genotypes could be determined for studied accessions whose S alleles were unknown or not known with certainty. As the newly isolated S8 haplotype seems to be frequent in the Eastern European region, its discrimination from SC will be necessary for the screening of European genetic resources used in apricot breeding.

3.2.3. Searching for the source of Sc:

In China, its centre of origin, apricot is self-incompatible. However, most European cultivars are self-compatible. In most cases, self-compatibility is a result of a loss-of-function mutation within the pollen gene *(SFB)* in the *SC* haplotype. Controlled pollinations performed by Halasz et al., (2007), revealed that the cross S8S9 × SCS9 set well, as expected, but the reciprocal cross did not. These authors analyzed apricot S8, S9 and SC haplotypes using a multi-approach methods including fruit set evaluation, pollen tube growth analysis, RNase activity assays, polymerase chain reaction (PCR) analysis and DNA sequencing of the *S-RNase* and *SFB* alleles. *SFB8* was revealed to be the first known progenitor allele of a naturally occurring self-compatibility allele in *Prunus* , and consequently *SC=S8′*.

The hypothesized pollen-part mutation within the *SC* haplotype was functionally verified by test crosses. As the SC cultivar SCS9 shares the S9 incompatibility haplotype with the SI cultivar S8S9, only the pollen grains carrying the S8 allele should overcome the incompatibility barrier. However, if *SFB*8 is a functional version of *SFB*C, this will recognize the Sc-RNase as self and fertilization will fail. The cross between the pollen parent S8S9 and the seed parent SCS9 proved incompatible, while the reciprocal was successful, confirming that SC is a pollen-part mutated form of S8.

Halász et al. (2005) showed that the SC-ribonuclease had the same isoelectric point as the S8-rnase in the SI cultivar 'Ceglédi óriás' (S8S9). Similar isoelectric points were presumed for SC and S7 by Alburquerque et al. (2002), but PCR and DNA sequencing failed to confirm their identity (Vilanova et al., 2005). In a study conducted recently by Halasz et al., (2007) they did detailed analyses to confirm the suspected identity of the apricot *SC* and *S8-rnases*. The *SC*- and *S8-rnase* alleles produced equally sized fragments in both PCRs, and the sequences of their first intron regions were identical. The amino acid sequences deduced from the partial - and S8-rnase cDNAs were also identical.

Cloning and sequencing of partial *SFB* alleles from S8S9, SCS9 and SCSC revealed that the nucleotide sequences of *SFB*8 and *SFB*C were identical except for a 358-bp insertion in the latter. Our results verified the position of the inserted sequence previously predicted by Vilanova et al. (2006) for 'Currot' (SCSC). Therefore, should be used as an informative synonym of SC. The premature stop codon is located near the beginning of the inserted sequence and results in a truncated F-box protein that

lacks the hva and hvb regions essential for the allelespecific recognition (Ikeda et al., 2004; Nunes et al., 2006). In the Japanese apricot *SFBf* allele, a 6.8-kbp insertion had a similar consequence (Ushijima et al., 2004). Sonneveld et al. (2005) suggested the presence of a general inactivation mechanism for non-self *S-RN*ases in the pollen tubes with *SFB* protecting self *S-RNases* from inactivation. Accordingly, the truncated apricot *SFBC*, by not recognizing the *S8-rnase* as self, cannot protect it from being degraded by the 26S proteasomes and pollen rnas will remain intact.

In our experiments we tried the reciprocal crosses to find out any probability for pollen-part mutation as a new source of self compatibility. Twenty reciprocal crosses which supported by following pollen tube growth experiments, all set fruits even poor. Yet, interestingly some evidences in our results are indicating the possibility of proposed source of self-incompatibility; mutation in pollen part determinant. The assigned S-genotype for MAYA which obtained based on the sequence homology analyses is *S2S9* both indicating self incompatibility behavior but we have phenotyped it as self-compatible. One possibility to explain this conflict is to consider non-functional version of one of S alleles (putatively S2) in the MAYA genotype. Thus the pollen part determinant can not recognize the same cognate S-RNase of pistil during self pollination which is entered beside the other S-RNase into the pollen tube, and the pollen tube continues to growth, which normally must be stopped. This allele is inherited from PORTICI, which is a self-compatible variety without Sc allele. Villanova et al., 2006 described a mutated S2 allele in the cultivar CANINO. The correspondence of these two S2′ alleles is supposed.

Chapter 4:

Conclusion

The study of the flower biology of apricot, has strong implications for the breeding program of this species, which has been developed at the same time. First of all, the knowledge of the factors limiting fruit set in an important number of commercial cultivars has oriented the selection of parents. Some cultivar-dependent characteristics, like style size, indicate that some cultivars would not be a good choice as parents in the breeding program. Other factors, like ovule immaturity at anthesis, are signs of bad adaptation of the cultivars to local climatic conditions and these, therefore, would also be a wrong parental selection. In those cases when such parents must be used, the knowledge of these characteristics is important in order to evaluate the seedlings, paying much attention to the possible segregation of these traits within the progenies in order to select the ones that have not inherited the undesirable characters. Determining the mode of inheritance of productivity traits improves the efficiency of breeding. Determining the inheritance of self-(in)compatibility and the parents' genotypes for this trait allows hybridizations to be planned which minimize or eliminate the production of self-incompatible seedlings. With the applied methodology, homozygous self-compatible cultivars can be easily identified, which will produce 100% self-compatible progeny regardless of the other parent's genotype. The number of publications in recent years indicates the interest in the different aspects of reproductive biology. This interest is, possibly, closely linked to the fact that this knowledge may avoid production failures and also allows the efficiency of the fruit breeding programs to be increased.

- Using PCR with S-allele-specific primers allows detection of the self-incompatible genotype in the first stages of plant development, and therefore allows rouging of undesirable seedlings straight after germination of the seeds. Specific

primers to amplify selectively the allele (or alleles) that determine self-compatibility are molecular markers for this trait with 100% efficiency, since they are located within the S locus. In apricot, these primers partially are developed but according to our findings are not sufficient. However, some recent papers on this species, and methodologies developed in related *Prunus* species, indicate that they will soon be available.

- It was found that the size polymorphism at 2nd intron is higher than 1st intron of *S-RN*ase gene in apricot, therefore designing and applying primer sets to amplify the 2nd intron would be more effective to discover the allelic variants at locus S.
SFB genotyping by SSCP can support the S-genotyping but often the sequencing of the PCR products is needed.

- The presence of different genetic sources of Self-compatibility in apricot has to be considered. About applicability of the *SFB*-based S-genotyping procedure, it is notable that The *S*-genotyping methods evaluated and used here to determine the *S* genotypes of cultivars in order to examine their right S-genotype and looking for *SC* allele origin will also be useful for the breeding, selection and characterization of apricot cultivars.

- This study provides evidence corresponding to the findings by previous researchers (Vilanova, 2006 & Halasz, 2007) who proposed and documented the origin of the pollen part mutated Sc and S2 haplotypes conferring self-compatibility trait in apricot. In both cases the self-compatibility appeared by mutation on the pollen determinant. For that reason it is better to combine the results of *S-RN*ase- and the new *SFB*-based PCR methods. In this way S genotypes was determined for 22 accessions whose S haplotypes were unknown or not known with certainty.

- *S-RNase* sequence data from various sources are as flash light to elucidate the putative origin and dissemination of self-compatibility in apricot conferred by the *SC* haplotype.
- Allelic variant is more greater and sometimes the polymorphism in size is not sufficient to specify a certain allele. Studying new varieties from different origins

presents new allelic variants. Attempts should be applied to clarify the synonyms for both *S-RNase* and *SFB* alleles.

- Referring to the databanks such as EMBL to find the corresponding sequence for new sequences sometimes is not useful as for several already identified *S-RN*ase and F-box genes the sequences are not deposited yet, in spite of reports in literatures confirming their identification.

- Finally self-incompatibility in apricot has to be well characterized both in segregating progenies and by molecular approaches. S locus seems to be very well conserved among *Prunus* species but mutations have to be verified by phenotyping accessions and cultivars case by case. Only in this way it will be possible to avoid mistakes as the case of NINFA erroneously indicated as *S1S2* in literature.

Bibliography:

1. Alburquerque, N., Egea, J., Pérez-Tornero, O. & Burgos, L. (2002): Genotyping apricot cultivars for self-(in)compatibility by means of RNases associated with *S alleles*. Plant Breed., 121: 343–347.
2. Anderson M.A., Cornish E.C., Mau SL., Williams EG, Hoggart R, Atkinson A, Bonig I, Grego B, Simpson R, Roche PJ, Haley JD, Penschow JD, Niall HD, Tregear GW, Coghlan JP, Crawford RJ, Clarke AE (1986): Cloning of cDNA for a stylar glycoprotein associated with expression of self-incompatibility in *Nicotiana alata*. Nature, 321: 38–44.
3. Andrés, M.V. & Durán, J.M. (1998): Self-incompatibility in Spanish clones of apricot (*Prunus armeniaca* L.) tree. Euphytica, 101: 349–355.
4. Aranzana, M.J.; García-MAS, J.; Carbó, J. and Arús, P. (2002): Development and variability analysis of microsatellite markers in peach. Plant Breeding, vol. 121, no. 1, p. 87-92.
5. Audergon, J.M., Guerriero, R., Monteleone, P. & Viti, R. (1999): Contribution to the study of inheritance of the character self-incompatibility in apricot. Acta Hort., 488: 275–279.
6. Bassi D. & J.M Audergon (2006), Apricot breeding: update and perspectives. ISHS Acta Horticulturae 701: XII International Symposium on Apricot Culture and Decline.
7. Ben-Njima, N. & Socias i Company, R. (1995): Characterization of some self-compatible almonds. I. Pollen tube growth. HortScience, 30: 318–320.
8. Beppu K, Yamane H, Yaegaki H, Yamaguchi M, Kataoka I, Tao R (2002): Diversity of *S*-RNase genes and *S*-haplotypes in Japanese plum (*Prunus salicina* Lindl.). J. Hortic. Sci. Biotech., 77: 658–664.
9. Beppu K, N. Komatsu, H. Yamane, H. Yaegaki, M. Yamaguchi, R. Tao and I. Kataoka, Se-haplotype confers self-compatibility in Japanese plum (Prunus salicina Lindl.), J. Hortic. Sci. Biotechnol. 80 (2005), pp. 760–764.
10. Bian, X-Y (2001): Ph.D. Thesis, The University of Adelaide
11. Bosˇkovic´, R., Tobutt, K.R., Batlle, I. & Duval, H. (1997): Correlation of ribonuclease zymograms and incompatibility genotypes in almond. Euphytica, 97: 167–176.
12. Bosˇkovic´, R. & Tobutt, K.R. (1999): Correlation of stylar ribonuclease isoenzymes with incompatibility alleles in apple. Euphytica, 107: 29–43.
13. Bosˇkovic´, R., Tobutt, K.R., Batlle, I., Duval, H., Martinez-Gomez, P. & Gradziel, T.M. (2003): Stylar ribonucleases in almond: correlation with and prediction of incompatibility genotypes. Plant Breed., 122: 70–76.
14. Brace, J., King, G.J. & Ockendon, D.J. (1993): Development of a method for the identification of S-alleles in *Brassica oleracea* based on digestion of PCR-amplified DNA with restriction endonucleases. Sex. Plant Reprod., 6: 133–138.

15. Brewbaker JL, Natarajan AT. (1960): Centric fragments and pollen part mutation of incompatibility alleles in Petunia. Genetics, 45: 699–704.
16. Broothaerts, W., Janssens, G.A., Proost, P. & Broekaert, W.F. (1995): cDNA cloning and molecular analysis of two self incompatibility alleles from apple. Plant Mol. Biol., 27: 499–511.
17. Broothaerts W, Keulemans J, Van Nerum I (2004): Self-fertile apple resulting from *S*-RNase gene silencing. Plant Cell Rep., 22: 497–501.
18. Burgos, L., Berenguer & T., Egea, J. (1993): Self- and cross compatibility among apricot cultivars. HortScience, 28: 148–150.
19. Burgos L., N. Alburquerque and J. Egea, (2004): Review. Flower biology in apricot and its implications for breeding, Spanish Journal of Agricultural Research 2 (2), 227-241
20. Chung K ., J. JiHae, and J. SangBouk, (2004): Self- and cross-compatibility of some apricot cultivars (Prunus armeniaca L.). Korean Journal of Horticultural Science & Technology. http://www.cababstractsplus.org/abstracts/Abstract.aspx?AcNo=20043178564
21. Cipriani, G.; Lot, G.; Huang, W.-G.; Marrazzo, M.T.; Peterlunger, E. and Testolin, R. (1999): AC/GT and AG/CT microsatellite repeats in peach (Prunus persica (L) Batsch): isolation; characterisation and cross-species amplification in Prunus. Theoretical and Applied Genetics, vol. 99, no. 1-2, p. 65-72.
22. Crane, M.B. & Lawrence, W.J.C. (1929): Genetical and cytological aspects of incompatibility and sterility in cultivated fruits. J. Pomol. Hort. Sci., 7: 276–301.
23. Cresti, M. & Went, van J.L. (1976): Callose deposition and plug formation in *Petunia* pollen tubes in situ. Planta, 133: 35–40. Currier, H.B. (1957): Callose substances in plant cells. Am. J. Bot., 44: 478–88.
24. CRPV, (2009): unofficial report by CRPV, Research Agency of Emilia Romagna Region.
25. Cruz-Garcia F, Hancock CN, McClure B. (2003): S-RNase complexes and pollen rejection. J. Exp. Bot., 54:123–30.
26. Currier, H.B., (1957): Callose substances in plant cells. Am. J. Bot., 44: 478-88.
27. Darwin C (1876): The effects of cross- and self-fertilisation in the vegetable kingdom. London: John Murray.
28. De Nettancourt, D., Devreux, M., Bozzini, A., Cresti, M., Pacini, E. & Sarfatti, G. (1973): Ultrastructural aspects of the selfincompatibility mechanism in Lycopersicon peruvianum Mill. J. Cell Sci., 12: 403–419.
29. De Nettancourt D (2001): Incompatibility and Incongruity in Wild and Cultivated Plants. Berlin/Heidelberg/New York: Springer-Verlag.
30. Di Sandro A, Serafini-Fracassini D., Del Duca S., Faleri C., Cai G., De Franceschi P., Dondini L., Sansavini S. (2008): Pollen Transglutaminase in Pear Self Incompatibility and Relationships with S-RNases and S-Allele Variability. Acta Horticolturae, 800: 423-430.
31. Dicenta, F. & García, J.E. (1993): Inheritance of selfcompatibility in almond. Heredity, 70: 313–317.

32. Dondini, L.& O. Lain & F. Geuna & R. Banfi & F. Gaiotti & S. Tartarini & D. Bassi & R. Testolin(2007): Development of a new SSR-based linkage map in apricot and analysis of synteny with existing Prunus maps. Tree Genetics & Genomes, 3:239–249
33. Doyle, J.J. & Doyle, J.L. (1987): A rapid DNA isolation procedure from small quantities of fresh leaf tissues. Phytochem. Bull., 19:11–15.
34. Entani T, Iwano M, Shiba H, Che F-S, Isogai A, Takayama S. (2003): Comparative analysis of the self-incompatibility (S-) locus region of *Prunus mume*: identification of a pollen-expressed F-box gene with allelic diversity. Genes Cells, 8: 203–13.
35. Egea J., L. Burgos, N. Zoroa and L. Egea, (1992): Influence of temperature on the in vitro germination of pollen of apricot (Prunus armeniaca L.), J. Hort. Sci. 67, pp. 247–250.
36. Egea, J., García, J.E., Egea, L. & Berenguer, T. (1991): Selfincompatibility in apricot cultivars. Acta Hort., 293: 285-293. Evans, N.A. & Hoyne, P.A. (1982): A fluorochrome from aniline blue: Structure, synthesis and fluorescence properties. Aust. J. Chem., 35: 2571–2575.
37. Egea, J. & Burgos, L. (1996): Detecting cross-incompatibility of three North-American apricot cultivars and establishing the first incompatibility group in apricot. J. Amer. Soc. Hort. Sci., 121:1002–1005.
38. Evans, N.A., Hoyne, P.A. (1982): A fluorochrome from aniline blue: Structure, synthesis and fluorescence properties. Aust. J. Chem., 35: 2571–2575.
39. Faust, M. 1989. Physiology of temperate zone fruit trees. Wiley, New York.
40. Feng, J., Chen, X., Wu, Y., Liu, W., Liang, Q. & Zhang, L. (2006a): Detection and transcript expression of S-RNase gene associated with self-incompatibility in apricot (*Prunus armeniaca* L.). Mol. Biol. Rep., 33: 215–221.
41. Franklin-Tong, V.E. (1999): Signaling and the modulation of pollen tube growth. Plant Cell, 11: 727–738.
42. Franklin-Tong, V.E. (2002): The difficult question of sex: the mating game. Current Opinion in Plant Biology, 5: 14–18.
43. Franklin-Tong VE, Franklin FC (2003): Gametophytic self-incompatibility inhibits pollen tube growth using different mechanisms. Trends Plant Sci., 8:598–605.
44. Gange, J.M., Downes, B.P., Shiu, S.-H., Durski, A. & Vierstra, R.D. (2002): The F-box subunit of the SCF E3 complex is encoded by a diverse-superfamily of genes in Arabidopsis. Proc. Natl. Acad. Sci. USA 99, 11519–11524.
45. Goldraij A, Kondo K, Lee CB, Hancock CN, Sivaguru M, Vazquez-Santana S (2006): Compartmentalization of S-RNase and HT-B degradation in self-incompatible *Nicotiana*. Nature 2006; 439:805-10.
46. Goldway M., T. Takasaki-Yasuda, J. Sanzol, M. Mota, A. Zisovich, R.A. Stern and S. Sansavini, (2009): Renumbering the S-RNase alleles of European pears (Pyrus communis L.) and cloning the S109 RNase allele, Scientia Horticulturae 119, pp. 417–422.
47. Golz JF, Oh H-Y, Su V, Kusaba M, Newbigin E (2001): Genetic analysis of *Nicotiana* pollen-part mutants is consistent with the presence of an S-

ribonuclease inhibitor at the S locus. Proceedings of the National Academy of Sciences, USA, 98: 15372–6.
48. Guerriero, R. & Bartolini, S. (1995): Flower biology in apricot: main aspects and problems. Acta Hort., 384: 261-271.
49. Halász, J., Ruthner, Sz., Békefi, Zs. & Pedryc, A. (2004): Sgenotype characterization of several Hungarian apricot varieties. 14th FESPB Congress, Krakkow, Acta Physiol. Plant., 26(3): 168.
50. Halasz J, Hegedus A, Herman R, Stefanovits-Banyai E, Pedryc A (2005): New self-incompatibility alleles in apricot (*Prunus armeniaca* L.) revealed by stylar ribonuclease assay and S-PCR analysis. Euphytica, 145: 57–66.
51. Halász, J. & Hegedûs, A. (2006): A critical evaluation of methods used for S-genotyping: from trees to DNA level. International Journal of Horticultural Science, 12 (2): 19–29
52. Halász J, Pedryc A, Hegedus A. 2007: Origin and dissemination of the pollen-part mutated SC haplotype which confers self-compatibility in apricot (Prunus armeniaca). New Phytol., 176(4):792-803.
53. Hauck NR, Yamane H, Tao R, Iezzoni AF (2006): Accumulation of nonfunctional S-haplotypes results in the breakdown of gametophytic self-incompatibility in tetraploid *Prunus*. Genetics, 172: 1191–8.
54. Herrero, M. & Hormaza, J.I. (1996): Pistil strategies controlling pollen tube growth. Sex. Plant Reprod., 9: 343–347.
55. Heslop-Harrison, J. (1975): Incompatibility and the pollen-stigma interaction. Ann. Rev. Plant Physiol. 26: 403–425.
56. Horiuchi H, Yanai K, Takagi M, Yano K, Wakabayashi E, Sanda A, Mine S, Ohgi K, Irie M (1988). Primary structure of a base non-speciWc ribonuclease from Rhizopus niveus. J Biochem, 103:408–418.
57. Huang S, Lee HS, Karunanandaa B, Kao TH (1994): Ribonuclease activity of *Petunia inflate* S proteins is essential for rejection of self-pollen. Plant Cell, 6: 1021–28.
58. Igic B, Kohn JR (2001): Evolutionary relationships among self-incompatibility *RNases*. P. Natl. Acad. Sci. USA, 98: 13167–13171.
59. Ikeda K, Igic B, Ushijima K, Yamane H, Hauck NR, Nakano R, Sassa H, Iezzoni AF, Kohn JR, Tao R (2004a): Primary structural features of the *S* haplotype-specific F-box protein, SFB, in *Prunus*. Sex. Plant Reprod., 16: 235–243.
60. Ikeda K, Watari A, Ushijima K, Yamane H, Hauck NR, Iezzoni AF, Tao R (2004b): Molecular markers for the self-compatible *S*4'-haplotype, a pollen-part mutant in sweet cherry (*Prunus avium* L.). J. Amer. Soc. Hort. Sci., 129: 724–728.
61. Ikeda, K., Watari, A., Ushijima, K., Yamane, H., Hauck, N.R., Iezzoni, A.F. & Tao, R. (2004): Molecular markers for the selfcompatible S4'-haplotype, a pollen-part mutant in sweet cherry (*Prunus avium* L.). J. Amer. Soc. Hort. Sci., 129: 724–728.
62. Ioerger TR, Gohlke JR, Xu B, Kao TH (1991): Primary structural features of the self-incompatibility protein in *Solanaceae*. Sex. Plant Reprod., 4: 81– 87.

63. Ishimizu T, Shinkawa T, Sakiyama F, Nakanishi T (1998b): Primary structural features of rosaceous *S*-RNases associated with gametophytic self-incompatibility. Plant Mol. Biol., 37: 931–941.
64. Janick J. & James N. Moore. (1996): Fruit Breeding; volume 1., Trees and Tropical Fruits. John wiley & Sons, Inc. New York.
65. Janssens, G.A., Goderis, I.J., Broekaert, W.F. & Broothaerts, W. (1995): A molecular method for S-allele identification in apple based on allele-specific PCR. Theor. Appl. Genet., 91: 691–698.
66. Jefferies, C.J. & Belcher, A.R. (1974): A fluorescent brightener used for pollen tube identification in vivo. Stain. Technol., 49:199–202.
67. J.wu, C. Gu, S. L. Zhang*, S. J. Zhang, H. Q. Wu and W. Heng, (2009): Identification of *S*-haplotype-specific *S-RNase* and *SFB* alleles in native Chinese apricot (*Prunus armeniaca* L.)
68. Kao TH, Tsukamoto T. (2004): The molecular and genetic bases of S-RNase-based selfincompatibility. Plant Cell, 16 (Suppl.): 72–83.
69. Kobel, F., Steinegger, P. & Anliker, J. (1938): Weitere Untersuchungen über die Befruchtungsverhältnisse der Kirschensorten. Landw. Jahrb. Schweiz, 52: 564–595.
70. Kobel, F., Steinegger, P. & Anliker, J. (1939): Weitere Untersuchungen über die Befruchtungsverhältnisse der Apfel- und Birnsorten. Landw. Jahrb. Schweiz, 53: 160–191.
71. Lai Z, Ma W, Han B, Liang L, Zhang Y (2002): An F-box gene linked to the selfincompatibility (S) locus of Antirrhinum is expressed specifically in pollen and tapetum. Plant Mol. Biol., 50: 29–42.
72. Lewis, D. & Crowe, L.K. (1954): Structure of the incompatibility gene. IV. Types of mutations in Prunus avium L. Heredity, 8: 357–363.
73. Lin, J.J. & Dickinson, D. B. (1984): Ability of pollen to germinate prior to anthesis and affect of desiccation on germination. Plant Physiol., 74: 746–48.
74. Linskens, H.F. & Esser, K.L. (1957): Über ein specifische Anfärbung der Pollenschläuche im Griffel und die Zahl der Kallosepfroplen nach Selbstung und Fremdung. Naturwiss, 44:16.
75. Martin, F.W. (1959): Staining and observing pollen tubes in the style by means of fluorescence. Stain. Technol., 34: 125–128.
76. Matthews, P. & Dow, K.P. (1969): Incompatibility groups: sweet cherry (Prunus avium). In: R.L. Knight (Ed.) Abstract Bibliography of Fruit Breeding & Genetics to 1965, Prunus, pp. 540–544. Commonwealth Agricultural Bureaux, Farnham Royal.
77. McClure BA, Haring V, Ebert PR, Anderson MA, Simpson RJ, Sakiyama F, Clarke AE (1989): Style self-incompatibility gene products of *Nicotiana alata* are ribonucleases. Nature, 342: 955–957.
78. McClure BA, Gray JE, Anderson MA, Clarke AE (1990): Self-incompatibility in Nicotiana alata involves degradation of pollen rRNA. Nature, 347: 757–60.

79. McClure BA, Mou B, Canevascini S, Bernatzky R (1999): A small asparagine-rich protein required for S-allele-specific pollen rejection in Nicotiana. Proc. Natl. Acad. Sci. USA, 96: 13548–53.
80. McCubbin AG, Kao TH (2000): Molecular recognition and response in pollen and pistil interactions. Annu. Rev. Cell Dev. Biol., 16: 333–364.
81. Mullis, K. B. & Faloona, F. A. (1987): Specific synthesis of DNA in vitro via a polymerase-catalyzed chain reaction. Methods Enzymol., 155: 335–350
82. Nasrallah JB (2005): Recognition and rejection of self in plant selfincompatibility: comparisons to animal histocompatibility. Trends Immunol., 26: 412–418.
83. Newbigin, E., Anderson, M. & Clarke, A.E. (1993): Gametophytic self-incompatibility systems. Plant Cell, 5: 1315–1324.
84. Nunes MDS, Santos RAM, Ferreira SM, Vieira J, Vieira P (2006): Variability patterns and positively selected sites at the gametophytic selfincompatibility pollen SFB gene in a wild self-incompatible *Prunus spinosa* (*Rosaceae*) population. New Phytologist, 172: 577–587.
85. Nyéki, J. & Szabó, Z. (1995): Cross-incompatibility in stone fruits. Horticultural Science, 28: 23–31.
86. Nyéki, J. (1996): Pollination and fertilization. In: Nyéki, J., Soltész, M., (Eds.): Floral Biology of Temperate Zone Fruit Trees and Small Fruits. Akadémiai Kiadó, Budapest.
87. Nyujtó, F., Brózik Jr., S., Brózik, S. & Nyéki, J. (1985): Fruit set in apricot varieties. Acta Agron. Acad. Sci. Hung., 34: 65–72.
88. O'Brien M, Kapfer C, Major G, Laurin M, Bertrand C (2002): Molecular analysis of the stylar-expressed Solanum chacoense small asparagine-rich protein family related to the HT modifier of gametophytic self-incompatibility in Nicotiana. Plant J., 32: 985–96.
89. Olden EJ, Nybom N. (1968): On the origin of *Prunus cerasus* L. Hereditas, 59:327–45.
90. Ortega, E., Egea, J., Cánovas, J.A. & Dicenta, F. (2002): Pollen tube dynamics following half- and fully-compatible pollinations in self-compatible almond cultivars. Sex. Plant Reprod., 15: 47–51.
91. Ortega, E. & Dicenta, F. (2003): Inheritance of self-compatibility in almond: breeding strategies to assure self-compatibility in the progeny. Theor. Appl. Genet., 106: 904–911.
92. Ortega, E. & Dicenta, F. (2004): Suitability of four different methods to identify self-compatible seedlings in an almond breeding programme. J. Hortic. Sci. Biotech., 79: 747–753.
93. Qiao H, Wang H, Zhao L, Zhou J, Huang J (2004): The F-box protein AhSLF-S2 physically interacts with S-RNases that may be inhibited by the ubiquitin/26S proteasome pathway of protein degradation during compatible pollination in *Antirrhinum*. Plant Cell, 16: 582–95.
94. Richman, A.D., Kao, T-h., Schaeffer, S.W. & Uyenoyama, M.K. (1995): S-allele sequence diversity in natural populations of Solanum carolinense (Horsenettle). Heredity, 75: 405–415.

95. Romero, C., Andrzej Pedryc, Verónica Muñoz, Gerardo Llácer, and María Luisa Badenes (2003): Genetic diversity of different apricot geographical groups determined by SSR markers , Genome 46: 244–252.
96. Romero C, Vilanova S, Burgos L, Martınez-Calvo J, Vicente M, Llacer G, Badenes ML (2004): Analysis of the S-locus structure in *Prunus armeniaca* L. Identification of S-haplotype specific S-RNase and F-box genes. Plant Molecular Biology 56: 145–157.
97. Sansavini S and L. Dondini, (2009): La genomica in frutticoltura: recenti acquisizioni per il miglioramento genetico, la produzione e la qualità del frutto. In Impatto della ricerca genetica e delle tecnologie avanzate sull'evoluzione dei sistemi agrari. Quaderni 2008-VI. Accademia dei Georgofili. Edizioni Polistampa, pp. 97-128.
98. Sanzol J, Herrero M (2002): Identification of self-incompatibility alleles in pear cultivars (*Pyrus communis* L.). Euphytica, 128: 325–331.
99. Sapir, G., Stern, R.A., Eisikowitch, D. & Goldway, M. (2004): Cloning of four new Japanese plum S-alleles and determination of the compatibility between cultivars by PCR analysis. J. Hortic. Sci. Biotech., 79: 223-227.
100. Sassa H., Hirano H, Ikehashi H (1992): Self-incompatibility-related RNases in styles of Japanese pear (*Pyrus serotina* Rehd.) Plant Cell Physiol., 33: 811–814.
101. Sassa H, Hirano H, Nishio T, Koba T (1997): Style-specific selfcompatible mutation caused by deletion of the S-RNase gene in Japanese pear (*Pyrus serotina*). Plant J., 12: 223–227.
102. Sassa H, Kakui H, Miyamoto M, Suzuki Y, Hanada T, Ushijima K, Kusaba M, Hirano H, Koba T (2007): *S* locus F-box brothers: Multiple and pollen-specific F-box genes with S haplotype –specific polymorphism in apple and Japanese pear. Genetics, 175: 1869-1881.
103. Sijacic P, Wang X, Skirpan AL,Wang Y, Dowd PE (2004): Identification of the pollen determinant of S-RNase-mediated self-incompatibility. Nature 29: 302–5.
104. Seiji T, Isaogai A (2005): Self incompatibility in plants, Annu. Rev. Plant Biol., 56: 467-489.
105. Socias i Company, R., Kester, D.E. & Bradley, M.V. (1976): Effects of temperature and genotype on pollen tube growth in some self-incompatible and self-compatible almond cultivars. J. Amer. Soc. Hort. Sci., 101: 490–493.
106. Socias i Company, R. & Alonso, J.M. (2004): Cross-incompatibility of 'Ferragnés' and 'Ferralise' and pollination efficiency for selfcompatibility transmission in almond. Euphytica, 135: 333–338.
107. Sonneveld, T., Robbins, T.P., Bosˇkovic´, R. & Tobutt, K. R. (2001): Cloning of six cherry sweet self-incompatibility alleles and development of allele-specific PCR detection. Theor. Appl. Genet. 102: 1046–1055.
108. Sonneveld, T., Tobutt, K.R. & Robbins, T.P. (2003): Allelespecific PCR detection of sweet cherry self-incompatibility (S) alleles S1 to S16 using consensus and allele-specific primers. Theor. Appl. Genet. 107: 1059–1070.

109. Sonneveld T, Tobutt KR, Vaughan SP, Robbins TP (2005): Loss of pollen-*S* function in two self-compatible selections of *Prunus avium* is associated with deletion/mutation of an *S* haplotype-specific F-box gene. Plant Cell, 17: 37–51.
110. Sutherland, B.G., Robbins, T.P. & Tobutt, K.R. (2004): Primers amplifying a range of Prunus S-alleles. Plant Breed., 123: 582–584.
111. Tao, R., Yamane, H., Sugiura, A. Murayama, H., Sassa, H. & Mori, H. (1999): Molecular typing of S-alleles through identification, chracterization and cDNA cloning for SRNases in sweet cherry. J. Amer. Soc. Hort. Sci., 124: 224–233.
112. Tao R, Watari A, Hanada T, Habu T, Yaegaki H, Yamaguchi M (2007): Self-compatible peach (*Prunus persica*) gas mutant versions of the S haplotypes found in self-incompatible Prunus species. Plant Molecular Biology, 63: 109–23.
113. Tsukamoto T, Hauck NR, Tao R, Jiang N, Iezzoni AF (2006): Molecular characterization of three non-functional S-haplotypes in sour cherry (*Prunus cerasus*). Plant Molecular Biology 62: 371–83.
114. Tufts, W.P. & Philp, G.L. (1922): Almond pollination. California Agricultural Experimental Station Bulletin, 346: 35.
115. Ushijima K, Sassa H, Dandekar AM, Gradziel TM, Tao R, Hirano H (2003): Structural and transcriptional analysis of the self-incompatibility locus of almond: identification of a pollenexpressed F-box gene with haplotype-specific polymorphism. Plant Cell, 15: 771–81.
116. Ushijima K, Yamane H, Watari A, Kakehi E, Ikeda K (2004). The S haplotype-specific F-box protein gene, SFB, is defective in self-compatible haplotypes of *Prunus avium* and *P. mume*. Plant J., 39: 573–86.
117. Vachun Z., (1981): Étude de quelques propriétés morphologiques et physiologiques du pollen d'abricotier. Germination et croissance des tubes polliniques a basses temperatures, Acta Hort. 85, pp. 387–417.
118. Vilanova, S., Romero, C., Llácer, G. Badenes, M.L. & Burgos, L. (2005): Identification of self-(in)-compatibility alleles in apricot by PCR and sequence analysis. Journal of the American Society for Horticultural Sciences, 130(6): 893–898.
119. Vilanova, S., Marı´a Luisa Badenes, Lorenzo Burgos, Jose´ Martı´nez-Calvo, Gerardo Lla´cer, and Carlos Romero (2006): Self-Compatibility of Two Apricot Selections Is Associated with Two Pollen-Part Mutations of Different Naturer. Plant Physiol. Vol. 142.
120. Viti, R., Monteleone, P., Guerriero, R. (1997): Incompatibility in apricot (Prunus armeniaca L.): methodological considerations. J. Hortic. Sci., 72: 961–970.
121. Wu, H.M., Wang, H. & Cheung, A.Y. (1995): A pollen tube growth stimulatory glycoprotein is deglycosilated by pollen tubes and displays a glycosilation gradient in the flower. Cell, 82: 393–403.
122. Yamane H.,Tao,R.,Murayama,H.and Lugiura A.(2000): Determining *S*-genotypes of several sweet cherry cultivars based on PCR-RFLP analysis. J. Hortic. Sci. Biotecnol. 75: 526-567.

123. Yamane H, Ikeda K, Ushijima K, Sassa H, Tao R (2003b): A pollen expressed gene for a novel protein with an F-box motif that is very tightly linked to a gene for *S*-RNase in two species of cherry, *Prunus cerasus* and *P. avium*. Plant Cell Physiol., 44: 764–769.
124. Yamane, H., Tao, R., Mori, H. & Sugiura, A. (2003b):Identification of a non-S RNase, a possible ancestral form of SRNases, in Prunus. Mol. Gen. Genomics, 269: 90–100.
125. Yamane, H., Ushijima, K., Sassa, H. & Tao, R. (2003c): The use of S haplotype-specific F-box protein gene, SFB, as a molecular marker for S-haplotypes and self-compatibility in Japanese apricot (Prunus mume). Theor. Appl. Genet., 107: 1357–1361.
126. Zhang L, Chen X, Chen X, Zhang C, Liu X, Ci Z, Zhang H, Wu C, Liu C (2007): Identification of self-incompatibility (S-) genotypes of Chinese apricot cultivars. Published online: 15 August © Springer Science+Business Media B.V. 2007
127. Zuccherelli S, Tassinari P, Broothaerts W, Tartarini S, Dondini L, Sansavini S (2002): S-allele characterization in self-incompatible pear (*Pyrus communis* L.). Sexual Plant Reproduction, 15: 153-158.

Summary

Self-incompatibility in apricot; New achievements and molecular aspects of S-locus allele segregation

Apricot (*Prunus armeniaca* L.), native to China and Central Asia and widely distributed over the world, has been attracted for its high drought resistance and obvious commercial prospect. The world production of apricot is 2,500,000 tons in 2007 (FAOSTAT, 2007).

Apricot breeding and production has been hampered by the trait of self-incompatibility (SI). Self-incompatibility of apricot varieties belonging to different eco-geographical groups been screened by several researchers. According to Guerriero and Bartolini 1995, the majority of the European varieties is self-compatible, however, most of the North-African, Middle-Eastern, Central Asian and Irano-Caucasian varieties are self-incompatible, but the number of self-incompatible varieties is increasing. From horticultural point of view there is a strong interest in self-fruitful cultivars.

Self-incompatibility (SI) is a mechanism whereby pistils reject pollen carrying the same S-allele. SI promotes genetic variability whilst at the same time, causing problems in crop and fruit production. Incompatibility in apricot is known to be of gametophytic type, the predominant form of SI system. It is based on interaction between the haploid pollen genome and the diploid pistil genome (McCubbin and Kao 2000). The incompatibility reaction, which occurs in the styles, involves two stages: pollen-pistil recognition, and subsequent rejection. The mechanism of rejection has not been fully explained, but callose is thought to play a key role. S-RNase has been shown to be involved in the gametophytic self-incompatibility of *Prunus* species. Apricot shows gametophytic self-incompatibility controlled by a single locus with several allelic variants. The allele of self fertility (*Sc)* is dominant over the incompatibility alleles (Burgos, 1995). Numerous *Prunus* S-alleles have been at least partially sequenced. They contain two very polymorphic introns (Igic and Kohn 2001). Consensus primers based on conserved regions, especially those flanking the polymorphic second intron, have been developed which distinguish S alleles on the

basis of size of PCR product, so that S-genotypes can be deduced from amplification patterns. To date, 30 S-haplotypes have identified in Eastern European and Central Asian cultivars and hybrids. As pollen determinant an F-box gene has been widely indicated and, actually, consensus primer for its amplification and characterization have been designed.

This research was organized at the Department of Fruit and Woody plant Sciences of Bologna University to clarify the right situation of a group of apricot varieties and accessions for their S-genotype and searching for new sources of self-compatibility trait in apricot.

In- field experiments: Self and cross pollination tests obtained during the years 2008 and 2009, the self-incompatibility of cultivars ROBADA, PINKCOT, GOLDRICH and HARGRAND is confirmed. Some new incompatible crosses were identified among the cultivars. The same experiments were also performed on PORTICI or BORA x MAYA (which is a seedling of the former two parental lines). Field crosses phenotyping was further investigated by optical microscopy by demonstrating the effective growth of pollen tubes in the pistils, eventually they ability to reach to the ovary

S-alleles Identification (RNase and F-box genes): We examined all the available primers specific for RNase and F-boxes for S-genotyping of 22 apricot accession or varieties: Altera, BO92639095, BO93622312, Bora, Cornia, Gheisi, Naderi, Kioto, Lillycot, Maya, Ninfa, Petra, Pieve, Pieve Tardiva, Pinkcot, Portici, Reale di Imola, Robada, S.castrese, Tardif de Valence, Yamagata 3 and Lito.

S-RNase specific primers were able to scan those genes both for the 1st or 2nd intron. S genotyping based on RNase amplification was confirmed by F-box amplification. In order to separate the bands related to each allele we used the SSCP (Single Strand Confirmation Polymorphism) and fortunately the linked bands separated and were more clear to identify. The primers; SFB and SFBc-R; which have generated according to the alignment of European apricot SFBs S1(AY587563), S2(AY587562) and S4(AY587565) (Romero et al. 2004), were able to identify eight F-box haplotypes (alleles), then it is concluded these primers set could be used for F-box typing in apricot. All the PCR results were confirmed by sequencing both of RNase

and F-Boxes. S-haplotypes for the 22 cultivars analyzed are reported in the table below:

The S-genotype assigned for the varieties studied in this work.

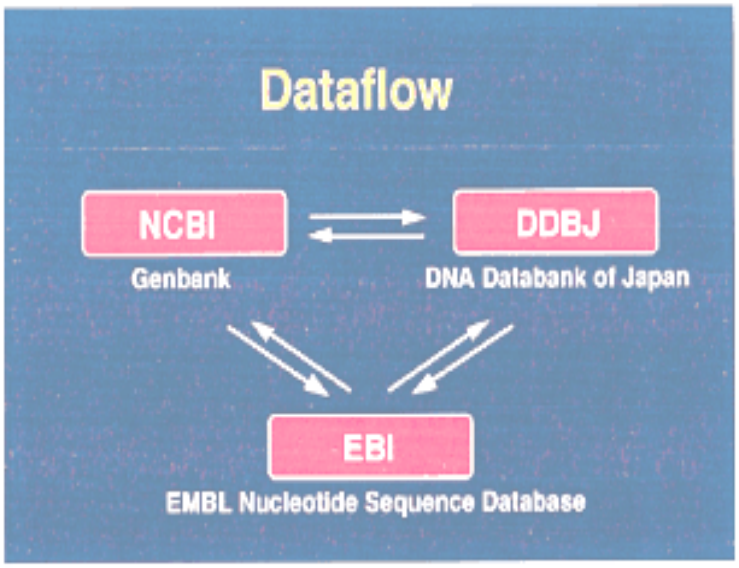
	Variety	S-genotype		Variety	S-genotype
1	ALTERA	S1SC	13	PETRA	S1S-
2	BO92639095	S1SC	14	PIEVE	S1SC
3	BO93622312	S1S-	15	PIEVE TARDIVA	S1SC
4	BORA	SCS9	16	PINKOT	S8S9
5	CORNIA	S1S-	17	PORTICI	S2(S17`)
6	HARCOT	S1S4	18	REALE	SCSC
7	GHEYSI	S2Sa	19	ROBADA	S8S22
8	NADERI	S13S14	20	S.CASTRESE	SCS-
9	KIOTO	SCS8	21	TARDIF DE VALENCE	SCS-
10	LILLYCOT	S8Sb	22	YAMAGATA 3	S8S-
11	MAIA	S9S2/8	23	LITO	SCS22
12	NINFA	SCS13	24	GOLDRICH	S1S2

Using PCR with S-allele-specific primers allows detection of the self-incompatible genotype in the first stages of plant development, and therefore allows rouging of undesirable seedlings straight after germination of the seeds. Specific primers to amplify selectively the allele (or alleles) that determine self-compatibility are molecular markers for this trait with 100% efficiency, since they are located within the S locus. In apricot, these primers partially are developed but according to our findings are not sufficient.

The presence of different genetic sources of Self-compatibility in apricot has to be considered. About applicability of the *SFB*-based S-genotyping procedure, it is notable that The *S*-genotyping methods evaluated and used here to determine the *S* genotypes of cultivars in order to examine their right S-genotype and looking for *SC* allele origin will also be useful for the breeding, selection and characterization of apricot cultivars.

This study provides evidence corresponding to the findings by previous researchers (Vilanova, 2006 & Halasz, 2007) who proposed and documented the origin of the pollen part mutated Sc and S2 haplotypes conferring self-compatibility trait in apricot. In both cases the self-compatibility appeared by mutation on the pollen determinant. For that reason it is better to combine the results of *S-RN*ase- and the new *SFB*-based

PCR methods. In this way S genotypes was determined for 22 accessions whose S haplotypes were unknown or not known with certainty.


Dataflow
NCBI
DDBJ
Genbank
DNA Databank of Japan
EBI
EMBL Nucleotide Sequence Database

Appendix A:

- **Apricot (*Prunus armeniaca*)*S-RNases* sequences in EMBL AND DDBJ**

DQ422947: Sc-RNase,5032bp

ACTATAGGGC ACGCGTGGTC GACGGCCCGG GCTGGTAAAG TCTTAACTGC AATAGCATCA
TGACGTCATT ATTACAGCTC GAAACATCCC ATGTTTACGT TCATGGAGAT GGTATTAACT
GCAATAGCAT CATGACGTCA TTATTAGAGC TCGAACATTC CATGTGGCCA TTGACTTTGG
CAGTTTGACG TCTTATCGCC TGAATAGCAC AAAAGAAATC ACTACTCTAC AACTCTAATA
TTAGAATTAT TTCTACCACC AAATCTAATT TTTAATCGGT TTGAAAACTT TACTACTTTG
TTCTAACATA TAGGATCCTT CCTTCCAATC GGAACTTTTT TTTTTTTATC TCCATGCACA
AAAGTTAGGG TGCAGATCAT TCATATTTGT GTAGGAAGCG TTATGTTAAA CTATAGGTAC
ACTAGTGGTT TGTGAGTGAA ATTTTGAGAG ATTCACTATT ATACTCAATA TAAGAGCCCA
AATTATAAAA ATATCTTATA TGAAATGAAC TTTAGAAATA CACCCAAAGC CCATTTACAA
TATAACAAAG AAGCTTTTAA CTTCTTATAA ATTACAAAAC TGCCATCAAT TTCTTAAAAC
AAGCCCAACT CCAAAATCTC ATGAAAATAC TCAAAACACT CAATAGGGCA TCAAAGTAAT
TTAATAATTA ATATTAAATT CAATAAGACC AGCTGTTATT TTTTGGATTT ACTTATAAAA
ATTCAATGGT GTATGGTTTA TCTATAATAT TAGTGCTAAA AATGAGTATA TAACTAAATA
TCTCTTAAAT TTTCTAATCA TGAGAGTTAG GCTTCACGTT GTCTTTCCAG TTTCTGTTTA
TTTGTTACGT GGCTTCCTCG TGTACTAATT TTTTTCTTTA ATCTTTTGAA TATGTTAAAA
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AY587562: S2-RNase, 6335bp

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TTTCCTTGTT CTTGCTTTCT TCTTGTGTTT CATAATGAGC ACTGGTGATG GTGGGTTGCA
TTACAATCTT TTGCTCTTTA TATGCCATGT ATGTGCATAT AATTTGCATT GAATTTTTCT
ACTTCTATTT TATGTGTGGA TAACTATTGT ATGTTTTCGA TGATAGGGAG GACTTATATA
GCGCACAACT TTCTTTACTC TGATAGTGTT GCAATAAGTA CAGTATTCAT CATTGGAAAC
CTTATTGAAG ATACCATTAA CCTTTTATCA CAATAATTTT GGCAGGAACT TATGACTATT
TCCAATTTGT GCAACAATGG CCACCGACCA CTTGCGGAGT TCGCGGGAAA CCTTGCTCCA
AACCCCGGCT ATTACAAAAT TTCACCATCC ATGGCCTATG GCCAAGTAAT TATTCAAACC
CAACGATGCC TAGTAATTGC AATGGGTCGA AATTTGAGGC AAGGAAAGTG GTATGTATTG
TTTTTATTTA TTTATTTATT AATAAATGTA TATATAGGTA TACCTATGTA TAGATCCCTT
TATAATAATG TATGTATCAT CTAATTAAAG GCATATAAAT TTTCCTGCAA CGGAATGCTT
GGTTAGCTCT TTATATAATT ATCCCCTTCC AATAAGATGG TGCCATCACC ATTTCATACA
AATTTTTCTA CTTAAAAACC TCCTAGTTGT TGGATATGGG TTTACAAATG GCACCATCTT
ATTGGAAAGG GGATGATTAT GTGGAGAGCT GACCTGGCAT TCCCGTGCCC ATGAAAGTTT
CTATCACTTT AGAGGAGAGA AAGTAGGTAT GGCAATGGAA CATCACTTAG GGTGTGTTCC
GGTTAGAGAT ATACCCGAAC ACCATGCTCG AAAATTTTCC GCAAACTCAT CCGTCCTTAT
ATTCGACTTC TAGTTTTTAT AACCCATTAG ATCGAAGCCC TGCAACTCGT GGAGAAATTT
GTCATCCCTA GGAAAAAGGG ATAACCTATC ATTTTGTACT TACGTAGACA TTCAGTATTT
AAAATATACT ATTTATGAAA GGATCAAAAT CAAAAGTCAA TTTAATACTC AGGTTCAATG
GGAAAAAGAG ATCCAAGAAT GAAAGTCTAA TCATTCCTTT AGCTTTACTC TTTTTTATTT
AGGAATTTCT GAATAAATAT TGCTTGTATG ACTCAGTACC CTCAATTGCG ATCCGATCTG
AAGATATCTT GGCCGGACGT GGAAAGTGGC AATGACACAA AATTTTGGGA AGGCGAATGG
AACAAACATG GAACATGTTC CGAACAGATA CTTAACCAAA TGCAATACTT CGAGCGATCC
CACGCAATGT GGACCTCGTA CAATATTACG AAAATCCTTA AAAATGCTTC AATCGTACCA
AGTGCGAAAC AAAAATGGAA GTACTCGGAC ATACTATCAC CCATTAAAAC AGCAACTGGA
AGAACGCCCC TCCTTCGTTG CAGAACTGAT CCTGCACTCC GTAATGTTCA GTTTTTACAT
GAGGTGGTGT TTTGTTATGG ATATAATGCG TTAAAGCAGA TTGACTGTAA TCGAACAGCA
GGATGCAAAA ATCAAGTTGA CATCGTGTTT AAATAAAATC TTATCTTTGT AAGTCATAAT
AAATTAGTAT TGACTAGTGT AATAAAAAAA AGTACTGTTT TCATATAAAA AAGGGTTTGT
AATGGTAGCA ATTACATTTG CATTTTTGAC AGGTTCTTTT CCACTTGCTT ACGCCATCCT
TGATTTTGAA AATGAAGCCA ATTAGACTCT TTTTTTTTTT CTTTTTCTAG AGAATTATAA
CAAGAGCATT ACGCAAGTTT AAGATCTACA CTCTC

AY587564:S4-RNase,1498 bp

AAAAAGACAA GGGTTTTTAT GATGATTTTT TTTTTTTTTT TTTATACATG TTATTTGGTT
CGTGAGAAAC TTGTGGGATT GAATTTACCT TTGCTCTTGC TCACAATTTA TAAGCGAGAG
ATTAATTGCG ATAGTACACA CCCCATACGC AACCTCCTAT ATAAACAGCA AACAAAGTGA
TTCTTAACTC AGAAAGCCTC TCCCATTCTG TTGTATTTCT TATTGCTTTA GTAGTATGGC
GATGTTGAAA TCGCCACTCG CTTTCCTTGT TCTTGCTTTT GCTTTGTTCT TGTGTTTCAT
TATGAGCACC GGTGGGTTGC ATTACAATCT TTTGCTCTTT ATATCCTATA CGCTAATTAG
CATGCATTGC ATTTTCCTAC TTATATTTTA TGTGTATTGG ATGATACAGG TGACATGCGC
TGTATTGAAT TAGCATTAAT CTTCTCACAA TAATTTTGGC AGGATCTTAT GTCTATTTTC
AATTTGTGCA ACAATGGCCA CCGGCCACCT GCATACGTAG CAAGAAACCT TGCTCCAAAC
ACCGGGCCTT ACAAAATTTC ACCATCCATG GCCTATGGCC AAGCAATTAT TCAAATCCAA
CGAGGCCCAG TAATTGCGTT GGGTCGCATT TTAACGAAAG TAAATTGGTA TGTATTGTTT
CATTTATTTT TCAATTACTC TAGCATTTTG TAAAAAAAAA TATATTACAA AATGATAGGT

ATTTTTTCAA TTACTTTATA ATTGAATATC TAACCATAGC TTGAGTTTCT ACTTTTTCTC
AAAAAATGTA TATATTGCTT GGATGTCTCA GTCCCCTCAA TTGATATCCA AATTGAGGAT
ATCTTGGCCT GACGTGGAAA GTGGCAATGA CACACAATTT TGGGAAGGCG AATGGAACAA
ACATGGTAAA TGTTCCCAAG AGAAGCTTAA CCAAATGCAA TACTTCGAGC GATCCCACGA
CATGTGGATG TCGTACAATA TCACGGATAT CCTTAAAAAC GCTTCAATCG TCCCACATCC
GACACAAACA TGGACTTACT CCGACATAGT TTCAGCCATT AAGAGTAAAA CTCAAAGAAC
ACCCCTCGTT CGTTGCAAAC GTGATCCAGC ACCGAATAAG AATGCGCCAA ACTCTCAGTT
GCTACATGAA GTGGTATTTT GTTATGAATA TAAAGCAAAA AAGCAGATTG ACTGTAATCG
AACAGCAGGA TGCTGGAATA ATGTTGACAT CAAGTTTCCT CCCTAAAATT ATAGCTAGCT
TTATAAGTCA TAACGAAGTA GTATGGTTTA GTATGGCACT AGTGTAATAA AAACAAAATG
AATGATTACA ATTTGGGAAG TCCGCCTTTC ATTTCTTTTA TTTTTTTAGT AAACGTATTA
CTTGTGATCC ATTGTCCATT TTGTTGTGAT TAATGAATTT ATTGTTCATT TTGTTGTGAT
TAATCAATTT TTGTCATATA CATCTAACAG TTGAACTTGT TCATGTCTAA CATTATCA

AY884212:S8-RNase, partial cds.927 bp

TATTTTCAAT TTGTGCAACA ATGGCCACCG ACTAACTGCA TAGTGCATAC GAAATGCTCC
AATCCCCGGC CATTACAAAT TTTCACCATC CATGGCCTAT GGCCAAGTAA TTATTCAAAC
CCAACGGTGC CCAGTAATTG CAATGGGTCA AAATTTGATG CAAGGAAAGT GGTACGTATT
GTTTTTTTTT TCTTTTTTTC CACGTACTCT TTAGCATTTA TACAAATTTA TAAATAAACC
CAATCCTAGA CATGTATTTA TAAAAAAACC CAAGCACTAT TTAAACATAG AAACACACCC
AAAATCAACT CCAATGTCAA CTTTTTGGGT GTGTTTATAA ATGGGTTTTG AGTGTGTGTA
ATGATGGGTT TTGGGTGTGT TTATATGTTT AAATAGTGCT TGGGTTTTTT TATAATTTCA
ACCCCCATTT TGGGTGTTTT AGTGAAACTC CCTATTGTAC TCCTAATGCC CTCAAAATGA
GAAGCACATG CGTCTCACAT TACTTTTTTT TTTTTTTTTT TCACGACAAT TTGAATGAAA
TTGTCTCGAA TCTAAATATA TTGTTTGGAT GTCTCAGTAC CCTCAATTGC AATCCAAACT
GAAGAAGTCT TGGCCTGACG TGGAAAGTGG CAATGATACG AAATTTTGGG AAGGCGAATG
GAATAAACAT GGTACATGCT CCGAACAGAC ACTTAACCAA TACCAATATT TCGAGATATC
CCACGACATG TGGTATTCGT TCAATATTAC AAACATCCTT AAAAACGCGT CAATCCTACC
AAGTGCGACA CAAACATGGA CCTATTCCGA CATAGTAGCA CCCATTAAAA CAGTAACTAA
AAGAACACCC CTCCTTCGTT GCAAACGTGA TCCAGCACGG AATAAGAGCC TGCCGAACTA
TCAGTTGTTA CATGAAGTGG TATTTTG

EF101909: S8-RNase, 418 bp

TACTTGTTCT TGGTTTTGCT TTCTTCTTGT GTTTCATTAT GAGCACTAGT GGTGGGTTGC
ATTACAATCT TTTGCTCTTT ATATCATATA TGCATATAAT TAGAATTACG AAGGAGAAGT
AGGCAGGAAA TGTCATTAAT TAGTACATAA CTTTCTTTGG ATGAGTTACT ATTTGGGAAT
TATTTTTCTG CATGGTATCT TTCGATTACT CTGGTAGTTG TTGAAATAAG TGCAGTATTC
ATCATTGGAA GCTAAAATGG TGTTCTTCCT GCATAAAATC CATTAACCTT CTCACAATAA
TTTTCGCAGG ATCTTATGTC CATTTTCAAT TTGTGCAACA ATGGCCACCG ACCACCTGCA
GAGTTCGCTG GAAACCTTGC TCCAAACCCC GGCCATTACA ATACTTCACC ATTCATGA

DQ269996:S9-RNase, 498 bp

TTCACAATTC ATGGCCTATG GCCAAGTAAC TATTCAAACC CAAGGAGGCC CAGTAATTGC
AATGGGTCGC GATTTAACTT TAGGAAAGTG GTATGTATTG TTTTTTTTTT TTTTTAACTT
ACTCTTTAGC ATTTAGTTCT AAAAAATTAG ATTGTCATCT GATGATAATA TACTTTCTTG
GTAGATAAAA TTGTAATGTT GGTTTTCTGG TTAGGAACAT TATTTTGCAT ATGTACCTAA
GTATAAAATT GGAACTAAAT TGAAAGGATA AAAATAAAAA TTCAATTTAA TACTCCCATT
TTATCCGATA ATGAAAATCT GTCCCTAATG TATTTCCCTT TTTTTTTTCT TTTTTTTTCT
TTCTAAAAAT ATGTTATATA TGGCTTGGAT GTCTCAGTAC CCTCAATTGC GAAATAAACT
GAAGATATCT TGGCCCGACG TGGAAGGTGG CAACGATACA AAATTTTGGG AAGGTGAATG
GAACAAACAC GGTACTTA

DQ269997:S10-RNase,445 bp

TTCACCATCC ATGGCCTATG GCCAAGTAAT TATTCAAACC CAACGATGCC CAGTAATTGC
AATGGATCGC GATTTAAGAA AGAGCTATCG GTAGGTATTG TTTCTTTTGT TTTTTGTTTT
TTTTCCATTT ACTCTTAAGC ATTTAGTTTT TAGGAAATTA GTTTGTCACC TGAAGATAAT
ATGCTTTTTT TCAATAAACC TTGCGAGATG GATAAAATTT TGATGCTAGT TCTCTGATTA
GGCACGTTAT TTTGACTATA TACATAAGGA CAGAATTACA AATGAGCAAT GCTAGGCATA
TCACATTTAC AAATGTGGTA TGACTAGCAT TTTTTATTAC AAATATATGT ATATTAGTAG
ATATATTAAT GTACATAATT TCAGTTATCA TATAAGATGG TATAGAATAT GGTAAAAATA

GCATTGGTAC CTAATAAAAT GGTAA

DQ868316:S11–RNase,464 bp

TCACCATTCA TGGCCTATGG CCAAGTAATT ATTCAAACCC AACGAAGCCC AGTAATTGCA
ATGGGACGAA ATTTGACGAC AGGAAAGTGG TATGTAATTT TTCTTTTTTC ACTTACTCTT
TAGCATTTAG GTTTTAGAAA ATTAGATTGT CGTCTGAAGA CAATATATAT ATATTGTAAG
TATATTTATA TCTGTATGTA TATTAATGAA TCTGCTCATC TAATTAAAGG ACCTTCCATT
TTGTACTTGC ACTATTGTAT ATAAATGAAT CAAAATCAAA ATTCAATTCA ATACTCAGAT
TTAATGGGGA AAAAACCAAT CTAATTATTT ATCAAATTAT GTATGTATTG CTTGGATGTC
TCAGTACCCT CATATGCGAT CCAAACTGAA GATATCTTGG CCCGACGTGG AAAGTGGCAA
TGATACAAAT TTTTGGGAAC GCGAATGGAA CAAACACGGC ACAT

DQ269998:S12–RNase,360 bp

TTCACCATTC ATGGCCTATG GCCAAGCAAT TATTCAAATC CAACGAGGCC CAGTAATTGC
GTTGGGTCGC ATTTTAACGA AAGTAAATTG GTATGTATTG TTTCATTATT TTTTCAATTA
CTCTAGCATT TTGTAAAAAA AAAATATATT ACAAAATGAT AGGTATTTTT TCAATTACTT
TATCCAAAAT TGAATATCTA ACCATAGCTT GAGTTTCTAC TTTTTCTCAA AAAATGTATA
TATTGCTTGG ATGTCTCAGT CCCCTCAATT GATATCCAAA TTGAGGATAT CTTGGCCTGA
CGTGGAAAGT GGCAATGACA CACAATTTTG GGAAGGCGAA TGGAACAAAC ACGGCACATA

DQ269995:S13–RNase 1259 bp

TTCACCATTC ATGGCCTATG GCCAAGTAAT TATTCAAACC CAACGATGCC CAGTAATTGC
AATGGGTCAA AATTTGAGGA CAGGAAAGTG GTATGTATTG TTTCATTATT TTTGTCACTT
ACCGTATTTA GTATTATAGT TCTTAATTAG AGAATCTACA ATGAGCTTCT TACACCATCT
ACAATGAACT CAATGATGCT CCCTTTATAT ATTTATGTAT CTGATCATCG AATTAAAGGA
CCTAGCTTTT CTATTGAAAG GACCAAAATC GAGATTCAAT TTAAAACTTC CTTCAATTTT
ATTATTATTG TTTCTGCCAA GTTATACCAC CATCTTTCTA AATAAAAAAG AAAAATCATT
GTAATGACTT TCATTGAGCC CCACCACCAG CAACACAATT GATTTTTACT TTATTTTCAT
CTTTTAAAGG AGTTGTGTTC AACCATCAAA TGTTTCTCTC TCTTCTCCTC TACTCAGTTC
TTCCTGTATC ATTTGGCTTT TGATCGAAGA AAGAGAAATA ATTCACAAGG AGCCATATAA
TAAACATAAA GCTGGGAAAC TAACAAACTA TATAATCTAA TAAAGAAAAT AGTAGAATTG
ACTTGATCCT AAAATTTCAA GTCTCATCTA ATGTGGCCCT TAAACTTTGT ATATCGTCAC
TTATGATGAA CTTGATCAAG GAAAGAGTGG AGGTATTTTG ACCCAAATCT GGGGCAAATC
CTCCCCAAAT TTGGAAGAAG TAATTAGATT ATCTAATCTA TCATCTTCTC CATATTGTAG
TGTTATATGG CCTCAAATAA ACTTGGGATT TCCAGGACAT GAGGAACCAG CAAAAGAGAT
AAGCCATATC GTATCTATAG ACGTTTGTCA ATGGCGGAAT CGCGCCCGTT CTGGTACTGC
AGCGATGACA GTGTTGCTGC TAGAATGATA TTATTAGGGT TTGTTTGTTA AACTAATTAA
TCAAATAAAT TTTAAAAACA TTATAAATAA ATAAAAGCTG ATTAAATTCA AATAGATTAT
GTGTCACACG GCATATGACA TGGATTGCCA TGTCACACAT CAGTTGCTAA CAAAAAGTTG
GTGTTTAAGT TACTGTTAAT GAAAAGTGTT GTTCAAAAAA GAAATTTTAT CACTGAAATT
TTTACTTTGA TGTTTCAGTC CCCTCAATTA CGATCCAAAT TGAAGAGGTC TTGGCCCGAC
GTGGAAAGTG GCAATGATAC AAAATTTTGG GAAGGTGAAT GGAACAAACA CGGCACATA

DQ870630:S14–RNase 492 bp

TCACCATTCA TGGCCTATGG CCAAGTAACT ATTCAAACCC AAGGAAGCCC AGTAATTGCA
ATGGGTCGCG ATTTAACTTT AGGAAAGTGG TATGTATTGT TTTTTTTTTT TAACTTACTC
TTTAGCATTT AGTTCTAAAA AATTAGATTG TCATCTGATG ATAATATACT TTCTTGGTAG
ATAAAATTGT AATGTTGGTT TTCTGGTTAG GAACATTATT TTGCATATGT ACCTAAGTAT
AAAATTGGAA CTAAATTGAA AGGATAAAAA TAAAAATTCA ATTTAATACT CCCATTTTAT
CCGATAATGA AAATCTGTCC CTAATGTATT TCCCTTTTTT TTTCTTTTTT TTTCTTTCTA
AAAATATGTT ATATATGGCT TGGATGTCTC AGTACCCTCA ATTGCGAAAT AAACTGAAGA
TATCTTGGCC CGACGTGGAA GGTGGCAACG ATACAAAATT TTGGGAAGGT GAATGGAACA
AACACGGCAC AT

DQ269999:S15–RNase,665 bp

TTCACCATTC ATGGCCTATG GCCAAGTAAT TATTCAAACC CAACGAAGCC CAGTAATTGC
AATGGGTCGC AATTTGATGC AAGGAAAGTG GTATGTATTG TTTCATTATT TTTTCACTTT
CTCTTTTAGT TTTTAGAAAA TTAGATTGTC ATATGAAGAT TTAAAAACAA AAACTACTTT
CAATAAGTCT TGGGTGCCAG ATAAATTTTT TGTGGTGGTT CTTAGCTTTT GTTTTGTTTT
TGGGGATGGT GGTTCTTAGT TAGACACATT ATTTTGAATA TATAAGTACA AAATGGTAAG

TATATTATAT TTATATGCAT ATTAATGTAC TTAAGAAAAT ATAATGGATC TGCTCATCTA
ATTATATGAC CTACCCCTTG ACCAAAAAAA ATGATGTACC ATTTTTGTAC TTTAAAAAAA
AAAGGGTATA ATATAAATCA AAATTCAATT TAATGAANCC AAAAAAAAAA AAAAAAATTT
ATTCAATAAT GAAAATCTAA TTATCACTTA TTAAAATTAC TTTTTCCAAA AATATGTATA
TATTGCTTGG ATTTGATGTC TCAGTCCCCT AAAATGCGAA TCAAGCTGAA GAAATCTTGG
CCAGACGTGG AAAGTGGCAA TGATACAAGA TTTTGGAAAG ACGAATGGAA CAAACACGGC
ACATA

DQ870632:S16-RNase 481 bp

TCACCATTCA TGGCCTATGG CCAAGTAATT ATTCAAACCC AAGGATGCCC AGTAATTGCA
CAGGGTCGCA ATTTAAGAAA CAGAATTTGG TATGTATTTT TTTCACTTAG TTTTTAGAAA
ATTAGATTGT TATCTGAAAA TAATATAAAC TTTTGAATAA ATTTTGGGTG TTACATAAAA
TTTTATGCTG GCACATATCG TAAAAATAAA GTTGGAAGTA CATTTATATA CGTATTTTAT
AATAATATAT CGAATCATTT AATAAATGGA TTTACCACTT TGTACTAGGG TATATAATGG
ACAGATTTAA TGAAAAACAG AAGTTCTTAT CCAATAATGA AAATCTAACT CTCCCTTCCG
TTTTTACTTT TTTCTCCTCA GTACCCTTAT ATGCAATCCA AACTGAAGAT TTCTTGGCCG
GACGTGGAAA GTGGGAATGA TACAAAATTT TGGGAAGGCG AATGGAACAA ACACGGCAGA
T

EU516388:S17-RNase 3127 bp

TCATTATGAG CACTGGTGAT GGTGGGTTGC AGTACAATCT TTTGCTCTTT ATATCCTATA
TGCATAAATC AGCATTGCAT TTTTCTACTT GTACTTTTTG TTCAGAGAAA CTATTTCAAC
TTTATTCACA TATTTTTCAT CCTTTATTCT AACGCACAAC TTTCTTTGGA TGAGTAATTT
GGGGATTGTT TTTCTGCATG TGCTCTTTTT CTATTTTCAT ACTCTTTTGT TTATTCTGAT
AATTGTTACA ACGTCTATTC ATCACAATAA TTTTGGCAGG ATCTTATGAC TATTTTCAAT
TTGTGCAACA ATGGCCACCG ACCAACTGCA GAGTTCGAAC CAAATGCTCC AACCCCCGGC
CATTACAATA TTTCACCATC CATGGCCTAT GGCCAAGTAA TTATTCAAAC CCGAAGATGC
CCAGTAATTG CATTGGGTCT CAATTTAACG AAAGTAGAGT GGTATGTATT TTTCATTATT
CTTTTCACAT GTATAGTTCT TAGAAAATTA GACTGACATT TGAAAATAGA ATAAACCACA
ATTGATGTTG GTCTCTGCTA GGCACGTATA TATATATAAT AATGAATCTG ATCATCAAAA
TGCAATGTTG ATCCTTTCAT TAAATCTTGA ATGTAACTAT CCCTTACGTT TTTACTGTTG
TCTCTCAAAA TATATTGCTT GGATGTCTCA GTACCCTTAT TTGCGCCCCA AACTGAAGAT
ATCTTGGCCA GACGTGGAAA GTGGCAATGA TACAAAATTT TGGGAAGGCG AATGGAACAA
ACATGGTACA TGTTCCGAAC GGATACTTAA CCAAATGCAA TACTTCCAGC GATCCCAAGC
AATGTGGAAA TCACACAATA TTTCTGAAAT CCTTAAAAAC GCTTCAATCG TACCACATCC
GACACAAACA TGGACCTACT CCGACATAGT ATCACCCATT AAAACAGCAA CTAAAAGAAC
ACCCCTCCTT CGTTGCAAAT ATGATAAGAA GACTCAGTTG TTACATGAAG TGGTATTTTG
TTATGGATAT AATGCGTTAA AGCACATTGA CTGTAATCGA ACCGCAGGAT GCCGAAATCA
ACAAGCCATC TCGTTTCAAT AAAATTATAA CTTTCTAATA AGTCATAATA AAGTAGTATG
GTCTAGTATG GTATTAAAGC AATAAAAACA AAATGAATGA TTGCACTTAC AATTTGGGAA
AACCCACCTT TTATTTTCTT CTTTTCTTTT ATGATTTATT GACATTTTTT AATTGATGTG
ACTATTCACT TTATCTGCTA CATAAGATGG TTTGAGAATG TGATATATAA ATATGGTAAA
AGTAGCATTA TTCTTTATTA AAATTATAGA GTGCCACGCA ATCATAGCAT TCTCTCATAC
TAAAAAAGAA GGCATTTGAT AGAACAATCA CATAATTTGA GCAAAAAGCT GCAATTGAAG
CAATGGCCAA TGAAGAAGGC ATTTGATAGA ACAATCATGT TCTTTCTAGC TCTTTCGGTT
TCTTCTCTTT CGTTCTTTTT TTGATCGAAA TCTGATTTCA TTAAAAGCCA AATAGGTAAG
CGAACAATAC ACAAAGCCAG TGGCATAAAA GGGCATCTAC AAACCCAGGA AGGAACAGCT
AAACTGAAAC TAAGTCTAAA ACAAAGTTCG CAAACCCGAC ACAAAAACAA AACCCCCCTA
AACAATTGGA GGGCAGGGAA GAACATGCTC AAAACAATTA GCCCAACACA GCTGTACACA
TAATTCAGAG GCCCAACCAA CAATCCAAAG GCCGAACTAA GCATAAAGCC CCACATCAGG
CACAAAGCCC CACATCCAAG CCCACCCTAT AGGACCACAT CCAAGGCAAA ATTTTGGTAA
ATCAATAGAG CCGACGTCGT ATCCATCCGA ATTCACAAGC TCCACCAAAA TCGAAGCCAC
CACAGCACCA AAACAAACGC AGCAGCCAAT CGGAGAACAA CCATGGGATG GAAATCATAA
CCCGAGACAG CCCATTTGAC ACCTTTAGCA TCATCTTTGG AAAAGAAACC GAAACAGCTA
GAAACCAAAA ATAATAATAA TAAGTTTGCA CATCTATTAA TAATTATTGA GTAAAACCAA
ACTTTGTATG TAAGTAATTG CAAAGAAGAA TTCGATTTCG TCATATTTCA TGACGGCCAA
CTTAATTCCT GTTTTAAGAA CTTCCTTGGA TTCGTAATTA CACAAATACA GATCTGCTAC
GCCCTTATCG AAATCTCTTA TTTCCATTAA CAGTTCATCA TCTATGCTAA TCCCGATTGT
AACGTTCCAT TCATCCCAAG GAAAAATAAA AGGACACAAT TGCTTCCACC GTTTTTCTTG
CAGAACCCAT AAGTCGACTT TTTCCATGCC ATCCTCCTCA CAACCATAAT ATCTACAAAG
CAAGCAAATT TGTCCCTTGT AAATGTCGAT ACATAAACCC CATGGACCGC AAATGGCATC
TGGTGCTATG AATTCTTCGA ATTCTTCACT GCCTGAATCA AAGGACATAA TGCTGAAGAT
GGGACCTTTC TCAATTATGT GATATGCTAC TCCATTAAAA AATGTATCCT TATGATTTTT

CCAAGTGCAT TTTAACCAAG GAGGAATTGC TTCAATCATC TTCCAAGAGT CTGTTCTGAG
ACTATAAACC TCAACCGCCA AGGCATTTTT GTTGGTACGC ATCATCCTTA CAGCCTTGTA
GTCATCAACC CCGGGGTGGA ATCCAAATTG GAGAGCAACA TAGCTAAATT TAATGTTAAT
GTTGGTGCTC GTTGGAGTGG TCCTAAATTT CCTGACCGAT GGGTTCCATA TGTGTATAGG
ACTATCGAAA TTCAATATCT CATCCGAAAT GCAAACTAAA CCATTGCTTG AACCATATAT
CCTATAATGC TCTGTGTTTT CTGAAGGATG GCTTAAATTG GAGAACTGCA CAAATGTTCC
ATTGGAAAAA AGTGACCATT GAAGTTCTTC TATATCATAT GGGTCATCAT TGTCGTTCTG
ACGTTCAAAG TTTGGGTGGT GGAGGCAAAG TAGATAGACA TGGGCATGTT TTGTGACATT
CCTATGAAGG TGTGTACTAA CAAAACCCGA GCTGCCAATC AAATCACTCC ACGACTTGCA
TGTACAC

DQ870634:S18-RNase,1337 bp

TCACCATTCA TGGCCTATGG CCAAGTAACT ATTCAAACCC AACGAAGCCC AGTAATTGCA
ATGGGTCGCG ATTTAACTTT ACGAAAGTGG TATGTTTTTT TTATTTTTAT TTTTACTTAC
TCTTTAGCAT TTACTTAGAA AATTAGACTG TCACTTGAAG ATAATATAAT TTTGTTAGGG
CCGTGTTATA ACCATTTCAG TTTTAGTTTT AAATTTTCAT TTTTCATACT AGAGTATAGA
GGCAAATGAG AGTGAGAATA TGAGTGAGGA TGAGAAGGAG GAGTAATAAG AGTGAAAACA
ATTTCAAATA GATTTCAGTT TTTAGTTTTT GTTTTCACTT TTGTTACTCC TCCCTCCCAC
CATCCTCTAT CACACGGACA GAGCCATGTA TGGGACTGAC ATGGCCCTAA CCCCCCAAAA
TTTTCCCCTA TAATATAATA TATTTATTTT ATGCTATGTA TATATTAGAG AAAATACTGT
TTTACTCTTA TGGCATCTCC AATATAGGCA TGCAACTCTT GAATTTTGCT CTATTTTCTT
GTTTGTTGTG GTTATTAAAT TATCTTTTGT TCCTTTTACC ATTATTGGTG CTTTAAAATA
TATGCCTTAT TATGTGTTTT CATTAGAGCT GGAAGTTTTG GCCCTTCAAA ATTATAAATT
CTAGCTCCGT CTTTGCTCTC TCATCTCTAC TTTCATTCTC CCTCTTTTTC CTGTATACTC
TTGCACAAAA AATAAAGACT AAAATCTAAA ACTAAAATGG TTATCAAATA GGCCAATAAA
ACCTTGGGTG CTAGATAAGA TGATGATTTT GGTTTGTAAG AGGGAAACAT ATTATATATA
TAAAGCTGTA ATACATTCAC ATTCGTTGTA ATGTACCTAT ATATAAGTAA TATATAATAA
TGGATCTGCT CATCTAATTA CAGGACCTCA TTAAAAAAAA AAACCAAAAC ACTCAATAAG
GTATCAAAGT AATTTAATAA TTAAAATTAA ATTCAATAGG ACCTGCTGTC ATTTTTCGGG
TTTTTGTTTG TTTGTTTTAT TTATAGAAAT TAAATGGTGT AAGGTTTATC TATATCATTA
GTGCTAAGAA TGAGTATATG ACTAAATATC TCAAATAAAA ATACAATTAG TATTCAGGTT
TAATTAAAAA AACAATCTTA TTCAGAAATA GAAATTTAAG CATCCCTTAA TTTTATAATG
TTTCTAACAA TATGTATGTA TTGCTTGGAT GTCTCAGTAC CCTCAATTGC GAACCAAACT
GAAGAAATCT TGGCCCGACG TGGAAAGTGG CAATGATACA AAGTTTTGGG AAAGCGAATG
GAACAAACAC GGCAGAT

EF133689: S19-RNase, 546 bp

TCACCATTCA TGGCCTATGG CCAAGTAACT ATTCAAACCC AACGATGCCC AGTAAATGCC
TTGGGTCGCA ATTTAAGGAG GAGAATTTGG TATGTATTGT TTGATTTTTA TATTTTTGCA
ATTACTCTTT ACGCATTTAG TTTTTTTTTA AAATAGATTG TCATCTGAAG CGAATATACT
TCTTTCAATA AACCTTGGGT AGTAGATAAA ATTTTGAACT TGGTCCTCTC GGTACATTAT
TTTGACTATG TACCTAAGTA CAAATGTATA ATCCTTTAAT TAAAGGGCCT ACCATTTTGT
ACTTACGTAT ATTCAAATAT TGTACCTAAT AAAAGGATCA AAATTAAAAT TCAATTTAAT
ATTCATGTTT AATTAACAAA ACAATCTTAT TCAAGAATGC AATATAACTA TACTTTAGTT
TTCACTTTTT TCCCAAAATG TGTCAGTCCC CTAAGTTGCT ATTGAAACTG AAGAGATCCT
GGCCGAACGT GGAAGGTGGC AACGACACAA GATTTTGGGA AGGGGAATGG AACAAGCACG
GCACAT

EF160078:S20-RNase,1934 bp

TCACCATTCA TGGCCTATGG CCAAGTAATT ATTCAAACCC ACGGAGGCCC AGTAATTGCA
ATGGGTCGCA ATTTAAGGAC GGGAAAGTGG TATGTATTGT TTCAAATATT TTTTTTTCTT
TCATATTGCT CTTAGAAAAT TAGATTGTCA TATGAAGATA ATACATTTTA TTTAATAAGC
CATGGTCTTG GATAAAATTT TGATGTTTGT CCTCTGCTAG GCACATTATT TTGAATCTCT
TTTGAAATAG GGGAAATATA TGGCTAAGTA CATTTATATT CATAGGTATT GATGTATATG
TCAAGTCAGA ATCGTGGGAA ATAAAAAAAA CACACATAAT TTGACACACT TTTTAATCTT
GAGATGAATC ACATTGAAAA TCATTTACCA TTTAAGAGAA ATGCATTTCT TTTATTACTT
CATTGAAAAC ACAAATACAT TTAATTATGT ATACTCTCTC ACTTCCCTTT ACTTATTATC
ATTTTATTCA AAAGCAACCT CCTTTATTAC TCACCACTTA AGAGTATCCT GACTCTCTAA
ACCACCTTAT TAGATAAAAT TTAAAAAGGA AGTGAGAAAA CTCATCTCTA ACCATGCTTT

TTATCCAGCT CATAGGGAGA CCTTTACGAG CTTTTAAATC TGAGGAGAGA GAATGGACCC
CTAATGGCTC CATATAATAT ATTGGTGCCT TATTTAATGA ATATTTTAAA CCTTTAATTA
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CATTTATGAC TCCCTAAAGA ATATGATGGA AGTTGAAATT TTATAGATAG CTCCTAAAAT
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EU570210:S21-RNase,1871 bp

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EU037262:S23-RNase,672 bp

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EU037263:S24-RNase,588 bp

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EU037264:S25-RNase,994 bp

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EU037265:S26-RNase,453 bp

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EU836683:S27-RNase,397 bp

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EU836684:S28-RNase,1353 bp

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EF185300:S29-RNase,1076 bp

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EF185301:S30-RNase,485 bp

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- ***S-RNases* sequences identified in this work**

Sequences: 27
Minimum Sequence Length: 179
Maximum Sequence Length: 418
Average Length: 349
Primer: SRc(F+R)

>ALTERA-C_400bp:S1

SRc(F)----------►
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◄----------SRc(R)

>ALTERA-A_353bp:Sc

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>BO92639095-A_353bp:Sc

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>BO92639095-B_400bp:S1

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> BO93622312-A_398bp:S1

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>Bora-A_353bp:Sc

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>CORNIA-C_400bp:S1

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>GHEYSI-D 376bp:S*

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>GHEYSI-E 324bp:S2

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>NADERI-A 399bp:S1

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>NADERI-B 202bp:

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> KYOTO-A 353bp:SC

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GCC

>LILLYCOT-D 267bp

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>LILLYCOT-E 354bp SC=S8

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>MAYA-E 245bp S*

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>MAYA-F 354bp SC=S8

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>NINFA-G 400bp S1?

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>NINFA-H 353bp: SC

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CATTAATTAGTACATAACTTTCTTTGGATGAGTTACTATTTGGGAATTATTTTTCTGCATGGTATCTTTC
GATTACTCTGATAGTTGTTGAAATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTCCTGCA
TAAAATCCATTAACCTTCTCACAATAATTTTCGCAGGATCTTATGTCTATTTTCAATTTGTGCAACAATG
GCC

> PETRA-F 397bp S1

CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCAGTGGTGGGTTGCATTACA
ATCTTTTGCTCTTTATATATCCTGTATATATGCATATAATCAGCATTACATTTTTCTAATTGTATTTTTT
GTTGAGAGAAACTATATTGTGAGTGTTCAATGATATATCACATGACATGCCGTCCACCCACTATTTTTCA
TTTAATCTAGCGCACAACTTTCTTTGGATGACTAAGTATTTGGGGATTGTTTTTCCAGCATGTTCTCTTT
TTATTTTCTCCTCTTTACTTTATTATGATAATTGTTGCAACTTGCATACGTGCAGTCTGTTCATCACAAT
AATTTTGGCGGATCTTATGATTATTTTCAATTTGTGCAACAATGGCC

>PETRA-G 180bp:S?

CTCGCTTTCCTTGTTCTTGCTTTTCTTTCCTTGTTCTTGCCTATTCCTCCCGTGCCAATGATTTCCTAAC
TGGGTTCATCTCTTTCCCTTGCTTTTCTTATATCTGGACAATCGTTTATTTTGTGAAGGAAGTCACATGG
AGATGGAGCTCAATTTTTATCAATTTGTGCAACAATGGCC

>PORTICI-A 328bp: S2

CTCGCTTTCCTTGTTCTTGCTTTTCTTCTTGTGTTTCATAATGAGCACTGGTGATGGTGGGTTGCATTAC
AATCTTTTGCTCTTTATATGCCATGTATGTGCATATAATTTGCATTGAATTTTTCTACTTCTATTTTATG
TGTGGATAACTATTGTATGTTTTCGATGATAGGGAGGACTTATATAGCGCACAACTTTCTTTACTCTGAT
AGTGTTGCAATAAGTACAGTATTCATCATTGGAAACCTTATTGAAGATACCATTAACCTTTTATCACAAT
AATTTTGGCAGGAACTTATGACTATTTCCAATTTGTGCAACAATGGCC

>PORTICI 329bp:S17

CTCGCTTTCCTTGTTCTTGCTTTTGCTTCTTGTGTTTCATAATGAGCACTGGTGATGGTGGGTTGCATTA
CAATCTTTTGCTCTTTATATGCCATGTATGTGCATATAATTTGCATTGAATTTTTCTACTTCTATTTTAT
GTGTGGATAACTATTGTATGTTTTCGATGATAGGGAGGACTTATATAGCGCACAACTTTCTTTACTCTGA
TAGTGTTGCAATAAGTACAGTATTCATCATTGGAAACCTTATTGAAGATACCATTAACCTTTTATCACAA
TAATTTTGGCAGGAACTTATGACTATTTCCAATTTGTGCAACAATGGCC

>ROBADA 419 bp S22

CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCACTGGTGGGTTGCATTACA
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TCTAGAGATATATTGTGTGTGTGATGATATATATATATATATATATATATATATATCAGGTAATGGAGGA
CTTGATCTAGCGCACACCTTTCTTTGGATGAGAAACTGTTTGGGATTTTTTTTTATTTTTTATTTCTGCA
TGGATTCTTTCGTTTACCGTGATAGTTGTTGCAATAAGTGCATTTGAAGCTAAAATTATGTTGTTTGAGA
TACCATCAACCTTCTCACAATAATTTTGGCAGGATCTTATGTCTATTTTCAATTTGTGCAACAATGGCC

>T.DI VALENCE-C 419 bp: S22

CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCACTGGTGGGTTGCATTACA
ATCTTTTGCTCTTTATATTATATCCTGGATGCATATAATTAGCATTGCATTTTTCTACTTCTATTTTATG
TCTAGAGATATATTGTGTGTGTGATGATATATATATATATATATATATATATATATCAGGTAATGGAGGA

CTTGATCTAGCGCACACCTTTCTTTGGATGAGAAACTGTTTGGGATTTTTTTTTATTTTTTATTTCTGCA
TGGATTCTTTCGTTTACCGTGATAGTTGTTGCAATAAGTGCATTTGAAGCTAAAATTATGTTGTTTGAGA
TACCATCAACCTTCTCACAATAATTTTGGCAGGATCTTATGTCTATTTTCAATTTGTGCAACAATGGCC

> LITO-A 353bp: SC

CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCACTAGTGGTGGG
TTGCATTACAATCTTTTGCTCTTTATATCATATATGCATATAATTAGAATTACGAAGGAGAAG
TAGGCAGGAAATGTCATTAATTAGTACATAACTTTCTTTGGATGAGTTACTATTTGGGAATTA
TTTTTCTGCATGGTATCTTTCGATTACTCTGATAGTTGTTGAAATAAGTGCAGTATTCATCAT
TGGAAGCTAAAATGGTGTTCTTCCTGCATAAAATCCATTAACCTTCTCACAATAATTTTCGCA
GGATCTTATGTCTATTTTCAATTTGTGCAACAATGGCC

- ***S-RNases*** **sequences identified in this work**

Sequences: 7
Minimum Sequence Length: 266
Maximum Sequence Length: 592
Average Length: 429
Primer: EMPc(2F+3R)

>NADERI; S14, 491bp

TCCAATTCATGGCCTATGGCCAGTAACTATTCAAACCCAAGGAAGCCCAGTAATTGCAATGGG
TCGCGATTTAACTTTAGGAAAGTGGTATGTATTGTTTTTTTTTTTTAACTTACTCTTTAGCAT
TTAGTTCTAAAAAATTAGATTGTCATCTGATGATAATATACTTTCTTGGTAGATAAAATTGTA
ATGTTGGTTTTCTGGTTAGGAACATTATTTTGCATATGTACCTAAGTATAAAATTGGAACTAA
ATTGAAAGGATAAAAATAAAAATTCAATTTAATACTCCCATTTTATCCGATAATGAAAATCTG
TCCCTAATGTATTTCCCTTTTTTTTTTCTTTTTTTTTCTTTCTAAAAATATGTTATATATGGC
TTGGATGTCTCAGTACCCTCAATTGCGAAATAAACTGAAGATATCTTGGCCCGACGTGGAAGG
TGGCAACGATACAAAATTTTGGGAAGGTGAATGGAACAAACACGGCAGAT

>LILLYCOT; S*[*], 266bp

ATGTGCCGTGTTTGTTCCTTCACGTTCCCAAAAGTCTGTATAATTGCCCTTTCCACGTTGGGC
CAAGATATCTTCAGTTTGGTTTCCAATCTAGGAGACTGAGACATGCAAGCAATAAAATATATT
GTGACGAACATACAAAAACTAAAGACTAACTAGAAAAACAAAACAGTCCGTACCAAACTATTG
TTAAATCGCGTTGGACTGCAATTAGCCACCCATGCGTTTTTTGAATGATTACTTGGCCATAGG
CCATGAATGGTGAA

>MAIA; S9, 583bp

TCCCATCCATGGCCTATGGCCAGTAATTATTCAAACCCAAGGTTGCCCAGTAATTGCATTGGG
TCGCAATTTAAGGGAATATTGGTATGTATTGGTTTTGTTTTTCACATGCTCTTTAGCTTTTAG
TTTTTACAAAATTTGATTGTCATATGGAGCTAATAAATTTTTTCACTAAACCTTGGCTTTTAG
GTAAAAGTTTGATGTTGGTCCTTTGGTTCGACACACTTGAAAATGTATCTAAGCACGAAATTG
TATATTTATATTATCCATGTATATATTTATAATATTGGATTTGATGATCTAATTAAGGGACTT
AAAATGTTGTACTTATGTATATATATTCAAAAAATTGTGCTCACTGAAAGGATATCAAGGTCA
AAATTCAATTTAATACTCAGGCTTAATGAAAAAAAAATCTTATCTAAAAATGAAAATGTAACT
ATCCCTAAGGTTGTACTTTTTTCTTTGGCCAAAAAATGAAAAAAAGGCTTTAACTTTTTTTCT
CAAACTATGTCTTAATTTTTTTTTCCCCAAACCTTTGCCTGCATGACCTCACTCCCCTTCAAT
TGCAGTCCCAACTGAA

>NINFA; S13, 592bp

TCCCATTCATGGCCTATGGCCAGTAATTATTCAAACCCAAGGAAGCCTAGTAATTGCAATGGG
TCACAATTTGACGCAAGGAAAGTGGTACGTATTGTTTCATTATTTTATATCTTACTCTTTGGC
ATTTAGTTTTTTAGGTTTTTTTATATATAGGAATTAGTGTTTAGAGAATTAGATTGTCATGTG
AAGATTTTAATAAATAAATAAACCTTTTTCAATAAGCCTTGGGTGTTATAGATTAAATTTTGA
TGTTGGTTCTTAGTTAGACACATTATTTTGAATATATAGTTAAGTACAAAATGGCAAGTACAT
ATTAATATACTTTCGAAAATATAATGGATCTGCTCATCTAATACCATTTTGTACTAATGCATA

[*] No high sequence similarity(homology) were found in DataBank.

TATGCAAAACATTGTACATCAAATTCTTTTTAAAGCAAGGCTATAATATATTATTGGAGATTA
AACTCAAAATTAATGCTCGGATTTAATGAGACAAAAAAACAATCTTTGTATTTTTAGTACAAG
CGACAATATAAATTACATGAGAATGAGTTTCTCACATACACATTATCATAGTGTCATAGAAGT
TGGAGTTTAAGACTAGAGATCTACA

>PINKCOT; S9/S17, 556bp

CACCATTCATGGCCTATGGCCAAGTAATTATTCAAACCCAAGGTTGCCCAGTAATTGCATTGG
GTCGCAATTTAAGGGAATATTGGTATGTATTGGTTTTGTTTTTCACATGCTCTTTAGCTTTTA
GTTTTTACAAAATTTGATTGTCATATGGAGCTAATAAATTTTTTCACTAAACCTTGGCTTTTA
GGTAAAAGTTTGATGTTGGTCCTTTGGTTCGACACACTTGAAAATGTATCTAAGCACGAAATT
GTATATTTATATTATCCATGTATATATTTATAATATTGGATTTGATGATCTAATTAAGGGACT
TAAAATGTTGTACTTATGTATATATATTCAAAAAATTGTGCTCACTGAAAGGATATCAAGGTC
AAAATTCAATTTAATACTCAGGCTTAATGAAAAAAAAATCTTATCTAAAAATGAAAATGTAAC
TATCCCTAAAGGTTGTACTTTTTTCTTTGGCCAAAAAATGAAAAAAGGCTTTAACTTTTTTTC
TCAAACTATGTCTTCATTTTTTTTTCCTCAAATATTTGCTTGGATGTCTCAC

>ROBADA; S8, 266bp

TCCCATTCATGGCCTATGGCCAAGTAATTATTCAAACCCAACGGTGCCCAGTAATTGCAATGG
GTCAAAATTTGATGCAAGGAAAGTGGTACGTATTGTTTTTTTTTTCTTTTTTTCCACGTACTC
TTTAGCATTTATACAAATTTATAAATAAACCCAATCCTAGACATGTATTTATAAAAAAACCCA
AGCACTATTTAAACACAGAAACACACCCAAAATCAACTCCAATGTCAACTTTTTGGGTGTGTT
TATAAATGGGTTTT

>YAMAGATA-3*, 334bp

TCCCATTCATGGCCTATGGCCAGTAACTACTCAAGCCCAACGAAGCCCAGTAGTTGCACTGGG
TCGGAATTTAAGGAATTGGTATGCATTATTTATTTATTTATCACATACTCTTTAGCATTTAGT
TTTTGTAACATTATTTTGGGTGTAAGATAAAATTTAAGGAATTGGTATGCATTACTTTTTTCA
ATAAACTTTGGGTATAAAATTTTGACGCTGGTTGTCTCGTTAAGCACATTATTTTGAATATAT
ACCTATCATTTTGTACTAATAATCTAATCAAAGGACTTACCAATTTGTAGTTACCTATATATT
CACAAAATCTCGTCCAAAT

* No high sequence similarity(homology) were found in DataBank.

Apricot (*Prunus armeniaca*) *SFB* sequences in EMBL

DQ422946:SFBc (SFB) gene, complete cds 3052 bp

---------- ---------- CGACGGCCCG GGCTGGTAAA TGACTCTTTG GCACAGTTCT
ATATGTTATT TTATTTTTGG TAACAGGCTC TGTATTTTAT TGTTTGACGC ACATGAACAG
TCATTTAATT CTGTTGGAAA TTTCATTCCC TTCCTTGCCT ATTCCCAACC TCAAAAGTGA
GTCGTTGGAC TTTCTGCAAA AATGAAAAGC ACTATTGTTT TCCTTGTTTT GTTATGATCA
ATATTTTGGA TGCTAACTTA TTTTGATTAT GATTTCTTCA GGATGACATT CACACTACGT
AAGAAAGAAA TCTTAATCGA CATCCTAGTA AGACTACCTG CAAAATCCCT TATTCGCTTT
CTGAGTACAT GCAAGTCGTG GAGTGATTTG ATTGGAAGCT CAATTTTTGT TAGCACACAC
CTTTGTAGGA ATGTGACAAA ACATACCCAT GTTTATCTAC TTTGCCTCCA CCACCCAAAT
TTTGAGCGTT TGGTCGACCC TAATGACCCA TATCTTAAAA AGGAATTTCA ATGGTCTCTT
TTTCCCAAAG AAACATTTGA GGAGTGCTAC AAACTAAGCC ATCCCTTAGG GATGACAGAA
CATTATGGGA TATATGGTTC AAGCAATGGT TTAGTTTGCA TTTCGGATGA GATCCTGAAT
TTCGATAGTC CTATACACAT ATGGAACCCA TCGGTTAGGA AATTTAGGGC CCTTCCAATG
AGCACCAACA TTAACATTAA ATTTAGCTGT GTTGCTCTCC AGTTCGGGTT CCACCCTGGG
GTTAATGACT ACAAGGCTGT AAGGATGATG CGTACCAATA AAAGTGCCTT GGCGGTTGAG
GTTTATAGTC TCAAAAGAGA CTCTTGGAAG ATGATTGAAG CAATTCCTCC TTGGTTAAAA
TGCACTTTGG AACATCATAG GGGTACGTTT TTCAGTGGAG TAGCATACCA CATCATTCAG
AAAGGTCCTA TGCTCAGCAT TATGTCATTC GATTCAGGCA GTGAAAAATT CGAAGAAATC
ATAGCACCAG ATGCCATTTG CAGTTTATGG GGGTTATATA TTGACGTTTA CAAGGAACAA
ATTTGCTTGC TTTTTATATG TTATGGGTGT GAGGAGGAGG GCATGGAAAA AGCTGACTTA
TGGGTTCTGC AAGAAAAACG GTGGAAACAA TTGAGTCCTT TTATTTAGAG ATATTTAGTG
ATATACCCAT TCTTAACACT AATATTATAG ATAAACCCTC CATCATTGAA TTTCTATAAA
CAAACCCAAA AAAAACCCAA AAAATGACAA CTGGCCTTAT TGAATTTAAT ATTGATTATT
AAATTACTTT AATGCCCTAT TGAGTGTTTT GGGTATTTTT ATGAGATTTT GGGGTTGGGC
TTGTTTTAAG AAATTTATGG TAGTTTTGTA ATTTCTAAGA AGCTAAAGCC TTTTTGTTAT
ATTGTAAATG GGTTTTGGGT GTGTTTATAA AGTTCATTTC ATATAGGGTA TTTTTATAAT
TTGGGTCTTC ATATTGGGTA TATTAGTAAA TCTCCCTTTT ATTTATCCTC CGGATTATTA
TTATTGTACA ACAGGGATTA GTATGGATAA CAAAATCTTA ATGCTAAGAG TAGATGACAT
TAGAGGCATA AGAAATCTGG ATTTATGTGA TTACGAATCC AACCAAGTTC TTGAAACAGG
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AGAAAGTTTG GTTTTACTCA ATAATTATTA AACAGATGTA TAAAGTTTGT TGTTATTTGT
TTTTCTTTCG GTTTATTTTG CAATATAATG ATGCTTGAGT CGAAAACTTA AATAGATAGT
TCGTATTATT AAACTAGAAT TTTCAGTAGG GCCCACAAGA AATTCCCACT TGTTGCAAAA
AATTATGGTC CTCAAATGAT GAGATTAGCG ATGGCTATGA GAATTTAAGG GTTCTGTGCT
AAATGAATAA CAAGGCATCA TAAATTAAAG TCTTCAATGA AAATATTATA AAAAAGAATT
GGACCATACT TCACTTTGTC TTTGCTATTG CTATATTAGA ATGAACTTAT TGATTTAAGG
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ACTTTCGATA AAATAAAAAA ATTTCTTATA AATACTAAAC TCATAGACTA TAGCTGATGG
ACAAAAAATA CTTGCAAAAT ATATGTTATA TTAATATTTT ATGTGTAATT AAAGGAATGT
TGTAGATTAT GTGCTTGATG AGATTCCTAT TTGGATTGCG GCTACCTTTG ATGATGATTT
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AATCCCCCCA TTTCAACACC AATAAAAAAA AGGAATGTTT TTTTTTCCTT CAGACGTGCA
AAGTTAAAAC AAATATATCC ATATATATAA AGCAAAAGGC CGAGAATGGT GAAACATTCA
AAATACCAGA AAATACTCTT GGTTAATTCA AACATTAAGA ATTGAAATTA TTGATTAAAT
TAGTATAATA TGGTAAATTC ACATTTTTTT CATATTAAAA AAAAATTAAA ATTAAAAACA
AAATCAAATA ATATGTGCTA CTTTTATAGA ACATAACTAC CTATGTTATC TTTTCTTAAT
TCTAAACATA AAATAAAAAA ATAAAAAATA AAACCAATTG GCACATGCTC ATGGCCAGTA
CTAATAACAT AAAATAATAC TAGCCCCTTG GCACATGCTC ATGCATGTGC CAATTGGTTT
TCTTTTTTCT TTTATTTTTA GTATTAAGAA AAGATAACAT GGGTAGTTAT GTTCCATAAA
AGTATGACTT ATTATCTGAT TTTGTTTTTA ATTTTACCAG CCCGGGCCGT CG

DQ422943:SFB1 gene, complete cds 2578 bp

AAAATAAAAC AGAAATCAGA TAATGGGTCC TATTTTTATG AAACACAATT ACCCATTATC
TTTTTTAATT CTAAAATAAA TAATATAAAA AATAAAACCA ATTGGCACAC GCTCACGCGT
GTGTGCCAAA GGGCTAGTAT TATATAAGCA TCGATCTCAG CAGGGTAAAT TAGTGAGTTA
CATTCATGAA TTGTTCATAC AATGTGCACC AACCCGTATA AAGAAATTGT CGATAAATTT
GTTTATATTA ATGGGCTTAG CATAAATTTC TTCCTATATA AATGGTGTCA CATAATTTTG
AACCAAAAAA AAAGGTGTCA CATAAAAGTT TGGGGCACAT AAAGAAAGTG TCCATGCATT
CCATAATATT TGAGAAAATT TCTTGATTTT GGGTTATATT GAAAGGACCA AACTCTTGAT

TCTAGTCCTA AGTTCCTAAC CTACCTCCCT CTCCAACTTG AGCTAGCTAA TTCCAAGAAT
ATATGAATTC TTTCTTTTCT GCAAGAAAAG GTACAATAAA AATAAAATTC AAAATAAACC
CCCTTTTCTT TTGTATCACT AAATGAACCT TTGGTGATGC TTTATTTTAG GATGCCAATT
GAATTACTTC CACATGACTC GTTCACACAG CTCTGTATAT TTTAATGCTT CCTTGGACTC
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TGCGCAAGAA AGAAATCTTA ACAGACATCC TAGTAAGACT ACCTGCAAAA ACCCTTGTTC
GATTTCTGTG TGCATGCAAG TCATGGAGTG ATTTGATTAA CAGCTCGAGT TTTATCACCA
CACAGCTTAA CAGGAATGTC ACCAAACACC TCCATGTCTC TCTACTTTGC CTCCACTACC
CCGATCTCAA ACGTCCTTTC GAGTTCTACG AAAACTACGA GGACTACCCA GATCTTAAAC
GAGAATTGGA ATGGTCACTT TTCTCCAACG AAACATTTGA GCATTGCTCC AAGTTAAACC
ATCCCTTGGG GATCAAGAAA GATTATAGGG TATATGGCTC AAGCAATGGC CTAGTTTGCA
TTTCGGATGA CAAATTGGAC ACCAAGAGCC CTATACACAT ATGGAACCCC TCGGTTAGGA
AATTTAGAAC CCTTCCAATG AGCACCAACG TTAAATTTCG CTATATTGCT CTCCAATTCG
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CATCCTCTTT GGTTAAAATG CACTTGGCAG AACCACAGGG GTACATTTTA TAATGGAGTA
GCATACCACA TTATTGAGAA ATTTCCTCTA TTCAGCGTTA TGTCATTCGA TTCGGGCAGC
GAAAAATTCG AAGAGTTCAT AGCACCGGAT GCCATTAGAT TTTGGTCGCT GCTGTATATT
GAGGTTTACA AGGATCAAAT TTGCTTGCTT TATTATTTGA GATTGTTTCA TTGTGAGGAA
GAAGGCATGT CACAAATTGA GTTTTGGGTT CTGCAAGAAA AACGATGGAA AGAAATGCGT
CCTTTTCTTT ATCCTTTCGA CTACTACAAT GTAGTTGGGT TCAGTATAGA TAATGAACTA
TTAATGGAGA GAAGTGGTTA TGGCAATGCT CTGTATTTGT GCAACTATGA ATCCAAGCAA
GGTCGTGAGA CAGGGATTGA GTTGGCCATC TCGAGAAATG ACCCTGAGCA GCTCTTGTTT
GTATTTACCT ACATAGAAAG TTTGATTTTA CTCAATTGTT AATTGGTGAA GAGATGTGTA
ACTTTTGGCA CGACCAGTTG GAGTTGGATT ATACATATCC AGATTATTAA TATTGACAGC
ATATTTTTTT TTGCTTTCCC ACCCATTACC ATGACAAATA AGGGTAATTA CTATTCACAT
GAGCCAATTT TATTTGTACA TATGAGATTA ACAAATAAAT AAATAAATTT GTTGGGTATG
TATAAAAAAG AAAATCTGAT GAATTTTTTG GTTATTTTTC AGAATTTTTT CAAATTTTTT
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GAAATGAGAT TTTCCTGCCC AGTGCCAAAA GCCACTGCTA GAACTGCACT AAGTTGTAAC
CCAAGTCCAA TATGGTATAA ATTGGACTTT TCAAAGCTAT GTTAAACAAT TTAGAATAGA
AGAGGGCATC AAATTGGGTG GGTCTGAGTA AGGGTGCGAG AAGAGAGCTA GGTTTTGAAT
AGGGGCAAAT GGGGTTTTGA TTGTAAACAA TTTTTTATTT ATTTTAGTCT TCTAGTGAAA
TTTCTCAAGG AGGTCATGGT TGTGTGTGTG TGATTCTTCG TGGTTGGTTG TCTGCTTTTT
GTCCATTGAT AACAAGGTGT GCCTAAGTAC AATAATATTG ACAGCATATC AAGATGAT

DQ422944:SFB2 gene, complete cds 2215 bp

AGATTGTTAC AACTTACATG GCTATCTTTC CGTGATGGTG GGAAATGCTT GGAGAGTAGT
GTTATGCTTG GGTTCCTATA TTGTTAGTTT AGTGGTTCAA TTTCATTTTA AATTAGATTT
CCATGAATAA AAATGTAATT ATTATTGTAT GTACGATATT TTCTTTTTAA ATTGAGCTAC
AAGGTCCTCA CACGATATTG CATCTGTATA TATAAATCAA AATGTAGAGA ATGATGAAAC
ATTCAAAATA CTGGAAAATG CATTAGTTAA GACAAACATT AAGAATTGAA ATTATTAATT
AAATAAACAT AATATGGTAA ATTCACACTT TTTTATATTT AAAAAAGTTT TAAAATAAAA
CAGAAATCAG ATAATGGGTC CTATTTTTAT GAAACACAAT TACCCATTAT CTTTTTTAAT
TCTAAAATAA ATAATATAAA AAATAAAACC AATTGGCACA CGCTCACGCG TGTGTGCCAA
GGGGCTAGTA TTATATAAGC ATCGATCTCA GCAGGGTAAA TTAGTGATTT ACATTCATGA
ATTGTTCATA CAATGTGCAC CAACCCGTAT AAAGAAATTG TCATAAATTT GTTTATATTA
ATGGGCTTAG CATAAATTTC TTCCTATATA AATGGTGTCA CATAATTTTG AACCAAAAAA
AAAGGTGTCA CATAAAGTTG GGGCACATAA AGAAAGTGTC CATGCATTCC ATAATATTGA
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CCTAACCTAC CTCCCTCTCC ACTTGAGCTA GCTAATTCCA AGAATATATG AATTCTTTCT
TTTCTGCAAG AAAAGGTACA ATAAAAATAA AATTCAAAAT AAACCCCCTT TTCTTTTGTA
TCACTAAATG AACCTTTGGT GATGCTTTAT TTTAGGATGC CAATTGAATT ACTTCCACAT
GACTCGTTCA CACAGCTCTG TGTATTTTAA TGCTTCCTTG GACTCTAAAT TTTCTAACTT
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CTTAACAGAC ATCCTAGTAA GACTACCTGC AAAAACCCTT GTTCGATTTC TGTGTGCATG
CAAGTCATGG AGTGATTTGA TTAACAGCTC GAGTTTTATC ACCACACAGC TTAACAGGAA
TGTCACCAAA CACCTCCATG TCTCTCTACT TTGCCTCCAC TACCCCGATC TCAAACGTCC
TTTCGAGTTC TACGAAAACT ACGAGGACTA CCCAGATCTT AAACGAGAAT TGGAATGGTC
ACTTTTCTCC AACGAAACAT TTGAGCATTG CTCCAAGTTA AACCATCCCT TGGGGATCAA
GAAAGATTAT AGGGTATATG GCTCAAGCAA TGGCCTAGTT TGCATTTCGG ATGACAAATT
GGACACCAAG AGCCCTATAC ACATATGGAA CCCCTCGGTT AGGAAATTTA GAACCCTTCC
AATGAGCACC AACGTTAAAT TTCGCTATAT TGCTCTCCAA TTCGGGTTCC ACCCCGGGGT
TAACGACTAC AAGGTTGTAA GGATGTTGCG CGTCCACAAA GATGATGCTT TCGCAGTCGA
AGTTTATAGT CTCAGCACAG ACTCTTGGAA GATGGTTGAA GAACATCCTC TTTGGTTAAA
ATGCACTTGG CAGAACCACA GGGGTACATT TTATAATGGA GTAGCATACC ACATTATTGA

GAAATTTCCT CTATTCAGCG TTATGTCATT CGATTCGGGC AGCGAAAAAT TCGAAGAGTT
CATAGCACCG GATGCCATTA GATATTGGTC GCTGCTGTAT ATTGAGGTTT ACAAGGATCA
AATTTGCTTG CTTTATTATT TGAGATTGTT TCATTGTGAG GAAGAAGGCA TGTCACAAAT
TGAGTTTTGG GTTCTGCAAG AAAAACGATG GAAAGAAATG CGTCCTTTTC TTTATCCTTT
CGACTACTAC AATGTAGTTG GGTTCAGTAT AGATAATGAA CTATTAATGG AGAGAAGTGG
TTATGGCAAT GCTCTGTATT TGTGCAACTA TGAATCCAAG CAAGGTCGTG AGACAGGGAT
TGAGTTGGCC ATCTCGAGAA ATGACCCTGA GCAGCTCTTG TTTGTATTTA CCTACATAGA
AAGTTTGATT TTACTCAATT GTTAATTGGT GAAGAGATGT GTAACTTTTG GCACG

DQ422945:SFB3 gene, complete cds 2216 bp

AGATTGTTAC AACTTACATG GCTATCTTTC CGTGATGGTG GGAAATGCTT GGAGAGTAGT
GTTATGCTTG GGTTCCTATA TTGTTAGTTT AGTGGTTCAA TTTCATTTTA AATTAGATTT
CCATGAATAA AAATGTAATT ATTATTGTAT GTACGATATT TTCTTTTTAA ATTGAGCTAC
AAGGTCCTCA CACGATATTG CATCTGTATA TATAAATCAA AATGTAGAGA ATGATGAAAC
ATTCAAAATA CTGGAAAATG CATTAGTTAA GACAAACATT AAGAATTGAA ATTATTAATT
AAATAAACAT AATATGGTAA ATTCACACTT TTTTATATTT AAAAAAGTTT TAAAATAAAA
CAGAAATCAG ATAATGGGTC CTATTTTTAT GAAACACAAT TACCCATTAT CTTTTTTAAT
TCTAAAATAA ATAATATAAA AAATAAAACC AATTGGCACA CGCTCACGCG TGTGTGCCAA
GGGGCTAGTA TTATATAAGC ATCGATCTCA GCAGGGTAAA TTAGTGATTT ACATTCATGA
ATTGTTCATA CAATGTGCAC CAACCCGTAT AAAGAAATTG TCGATAAATT TGTTTATATT
AATGGGCTTA GCATAAATTT CTTCCTATAT AAATGGTGTC ACATAATTTT GAACCAAAAA
AAAAGGTGTC ACATAAAGTT GGGGCACATA AAGAAAGTGT CCATGCATTC CATAATATTG
AGAAAATTTC TTGATTTGGG TTATATTGAA AGGACCCAAC TCTTGATTCT AGTCCTAATT
TCCTAACCTA CCTCCCTCTC CACTTGAGCT AGCTAATTCC AAGAATATAT GAATTCTTTC
TTTTCTGCAA GAAAAGGTAC AATAAAAATA AAATTCAAAA TAAACCCCCT TTTCTTTTGT
ATCACTAAAT GAACCTTTGG TGATGCTTTA TTTTAGGATG CCAATTGAAT TACTTCCACA
TGACTCGTTC ACACAGCTCT GTGTATTTTA ATGCTTCCTT GGACTCTAAA TTTTCTAACT
TATTTTTCTT ATGATTTTCT CTTCGTGAGA TGGCATCCAC CACATTACGC AAGAAAGAAA
TCTTAACAGA CATCCTAGTA AGACTACCTG CAAAAACCCT TGTTCGATTT CTGTGTGCAT
GCAAGTCATG GAGTGATTTG ATTAACAGCT CGAGTTTTAT CACCACACAG CTTAACAGGA
ATGTCACCAA ACACCTCCAT GTCTCTCTAC TTTGCCTCCA CTACCCCGAT CTCAAACGTC
CTTTCGAGTT CTACGAAAAC TACGAGGACT ACCCAGATCT TAAACGAGAA TTGGAATGGT
CACTTTTCTC CAACGAAACA TTTGAGCATG GCTCCAAGTT AAACCATCCC TTGGGGATCA
AGAAAAATTA TAGGGTATAT GGCTCAAGCA GTGGCCTAGT TTGCATTTCG GATGACAAAT
TGGACACCAA GAGCCCTATA CACATATGGA ACCCCTCGGT TAGGAAATTT AGAACCCTTC
CAATGAGCAC CAACGTTAAA TTTCGCTACA TTGCTCTCCA ATTCGGGGTT CCACCCCGGG
GTTAACGACT ACAAGGTTGT AAGGATGTTG CGCATCCACA AAGATGATGC TTTCGCAGTC
GAAGTTTATA GTCTCAGCAC AACTCTTGGA AGATGGTTGA AGAACATCCT CTTTGGTTAA
AATGCACTTG GCAGAACCAC AGGGGTACAT TTTATAATGG AGTAGCATAC CACATTATTG
AGAAATTTCC TCTATTCAGC GTTATGTCAT TCGATTCGGG CAGCGAAAAA TTCGAAGAGT
TCATAGCACC GGATGCCATT AGATATTGGT CGCTGCTGTA TATTGAGGTT TACAAGGATC
AAATTTGCTT GCTTTATTAT TTGAGATTGT TTCATTGTGA GGAAGAAGGC ATGTCACAAA
TTGAGTTTTG GGTTCTGCAA GAAAAACGAT GGAAAGAAAT GCGTCCTTTT CTTTATCCTT
TCGACTACTA CAATGTAGTT GGGTTCAGTA TAGATAATGA ACTATTAATG GAGAGAAGTG
GTTATGGCAA TGCTCTGTAT TTGTGCAACT ATGAATCCAA GCAAGGTCGT GAGACAGGGA
TTGAGTTGGC CATCTCGAGA AATGACCCTG AGCAGCTCTT GTTTGTATTT ACCTACATAG
AAAGTTTGAT TTTACTCAAT TGTTAATTGG TGAAGAGATG TGTAACTTTT GGCACG

AY587565:SFB4 gene, partial cds. 2031 bp

ATCATGATTA TTAAAAGAAA ATGTCAGTAC CATGATATTT TGGTTCCTTT TTTTTCTTTT
AGGAGAACTT TACGTTTTTT TACATTACCG ATGATCATTT TCTCCCCTTT TGACCCAAAA
AAAGGATCAT TTTTCTCTCT GTCATGTGGT GGTGCTTTAT TCTAGGACGC CAAATTACTT
GGCCGCTCTT TTATCCATTC AATAACGTTC AGCCAGGATC TTAATCCTTT TTTACTCCTG
AGAAGCTGAC CTCAAATGCA GTTCTTTCTT CCCTTTTGTT TTCCTTTTCT TTTCTTTTCT
TTTCTTGGAG GTTATAAATA TATAGTTTCA CATGACTAAT GTCACGGAGC TTTATATTTT
ATTGTTTGAC GCACACGAAC AGTAATTTAT TTCTGTAGGA AATTTATTGC TTTCCATTGC
CCATACCAAC CTCAGAAGTA AGTCATTGGT TCTGCAAAAC TAAAAGCACC ATTGTCTTCC
TAACTTTGTT TTGCAACGAT CCATACTTTA GATTCTAACA TATTTTCGTT ATGAGTTTCT
TCAGCATGAC ATTCACTCTA TGTAAGAAAG AAATTTTAGT CGACATCCTG GTAAGACTGC
CTGCAAAATC TCTCGTTCGG TTTATGTGTA CATGTAAGTC ATGGAGTGAT TTGATTGGCA
GCTCCAGTTT TGTTAGCACA CACCTTAATA GGAATGTCAA TAAACATGCC CATGTATATC
TACTTTGCCT CCACCATCCA AATTTTGAAT GTGTGGTGGA CCCTGATGAC CCATATTTAG
AAGAAGAAGT TCAATGGTCT CTTTTTCCCA ATGAAACATT TGAGGAATGC TCCAAGTTAA
ACCATCCCTT AGGGAGCACA GAACATTATG GGATATATGG CTCCAGCAAT GGTTTAGTTT
GCATTTCGGA TGAGATATTG AATTTCATAG TCCTATACAT ATATGGAACC CATCAGTTAG

GAAACTTCGG ACCCTTCCAA TAAGCACCAA CATCATTAAA TTTAGCCATG TTGCTCTCCA
GTTCGGGTTC CATCCCGGGG TTAATGACTA TAAGGCTGTA AGGATGATGC GTACCAACAA
AAATGCCTTG GCGATTGAGG TGTATAGTCT TAGAACAGAC TCTTGGAAGA TGATTGAAGC
AATTCCTCCT TGGTTAAAGT GCGCTTGGCA GCATTATAAG GGTACATTTT TCAATGGAGT
AGCATACCAT ATCATTGAAA AAGGTCCTAT ATTCAGCATC ATGTCCTTCG ATTCAGGCAG
TGAAGAATTT GAGGAATTCA TAGCACCAGA TGCAATTTGC CGTCCATCTG AGGTATGTAT
TGACGTTTAC AAGGAACAAA TTTGCTTGCT TTTTGGATTT TATCGTTGTG AGGAGATGGG
CATGGATAAA ATTGACTTGT GGGTTCTGCA AGAAAAACGG TGGAAACAGT TGTGTCCTTT
TATTTATCCA TGGGGTTTTT GTTATCGTAT AACCGGGATT AGTATAGATA ATGAACTCTT
AATGGGAAGA AGAGATTTCT TAAAGGGCGT AGCAGATCTC TATTTGTGTA AGTACGAATC
CAAGCAGGTT CTTGAAACAG GAATTAAGTT GGGCATCATG ACATATGGGG AAATCGAATT
CTTGTTTTCA TTTACTTACA TGGAGAGCTT GGTTTTACTC AATTAATTAG TAACATATAT
ATAAAGTTTG GTTTTTCTTC CGCTTTCTTT TGCAAGGCTG GTAGTTGAAC AAACACTTAA
AAATAGATGG TTTCTGTACT CCTTAATTAT GACCCTTGTA GAAATTAATC CCCTAAACTA
AAAAAGTATT ATATTTAATA CCCGAATTAA TTAAAACGGC CAATTTAACT CTTATTGTCA
AAATTTTCTA ATTTCATATA TTTTTTCGTT TATAAAGACA CGTGTCACGT GACTTACTAT
TTGAATTTTT TATTATTATA TTTAAAAACT AAAATATTGA AAAAACAAAC AAATAAAATA
ACTAAAAAAA TAAAAATTGG TTCTTTGCCG TCAATCGGTG GCTGCAATGA T

EU652883: SFB8 gene, partial cds 1083 bp

ATGGCATTCA CACTACGTAA GAAAGAAATC TTAATCGACA TCCTACTAAG ACTACCTGCA
AAATCCCTCA TTCGGTTTCT AAGTACATGC AAGTCATGGA GTGATTTGAT TGGCAGCTCG
AGTTTTGTTA GCACACATCT TAATAAGAAT GTCACAAAAC ATGCCCATGT CTATCTACTT
TGCCTCCACC ACCCAAATTT TGAATGTGTG ATCGACCCCG ATGACCCATA TTTAGAAGAA
GAACTTCAAT GGTCTCTGTT TTCCAATGTA ACATTTGAGA AGTGCTCCAA GTTAAGCCAT
CCCCTAGGAA GCACAAAACA TTATGGGATA TATGGTTCAA GCAATGGTTT GCTTTGCATT
TCTGATGAGA TATTGAATTT CGATAGTCCA ATACACATAT GGAACCCATT GGTTGGAAGA
TATAGGACCC CTCCAATGAG CACCAACATT AACATTAAAT TTAATTATGT TGCTCTCCAA
TTCGGGTTTC ACCCTGGGGT TAATGACTAC AAGGCTGTAA GGATGATGCG TACCAACAAA
GATGCCTTGG CGGTTGAGGT TTATAGTCTT AGAACAGACT CGTGGAAGAT GATTGAAGCA
ATTCCTCCTT GGTTAAAATG CACTTGGCAG CATCATATGG GTACATTTTT TAATGGAGTA
GCATACCATA TTATTGAGAA AGGTCCCATA TTCAGCATTA TGTCCTTCGA TTCAAGCAGT
GAAGAATTTG AAGAGTTCAT AGCACCAGAT GCCATTTGCA GTTCATGGAG GTTATGCATC
AGCGTTTACA AGGAACAACT TTGCTTGCTT TTTGGATTTT ATGGTTGTGA GGAGGAGGAC
ATGGAAAAAC TTCTCTTATG GGTTCTGCAA GAAAAACGGT GGAAACAATT GTGTCCTGTT
ATTTATCCTG AAGGTAATTG TCGTCATATT CTCGGAATTA GTATAGATAA TGAACTCTTA
ATGACAGAAA GAGGTTTCGA TACCGGCATA GCAGATCTGT ATCTGCGTAA TTATGAATCC
AAGCAAGTTC TTGAAACAGG AATTAAGTTG GCCGTCTTGA AATATGACGA AATCGAATTC
GTG

EU935588: SFB9 gene, partial cds. 1056 bp

GAAATCTTAA TCGACATCCT AGCAAGACTA CCTGCAAAAT CCCTCGTTCG GTTTCTGTGT
ACATGCAAGT CATGGAGTGA TTTGATTAGC AGCCCGAGTT TTGTCAGCAC ACACCTTTAT
AGGAATGTCA CAAAACATGC CCATGTCTAT CTACTTTGCC TTCACCACCC GAATTTTGAA
TGTGTGGTCG ACCCTGATGA CCCATATTTG GAAGAAGAGC TTCAATGGTC TCTTTTTTCC
AATGAAACAT TTAAGCTGTG CTCCAAGTTA AGCCATCCCT TAGGGAGTAC AAATCGTTAT
GGGATATATG GTTCAAGCAA TGGTTTAGTT TGCATTTCGG ATGAGATACT GAATTTCGAT
AGTCCTATAC ACATATGGAA CCCATCGGTA AAGAAATTTA CGAGCCCTCC AATGAGCACC
AACATTAACG TCAAATTTAC CTATGTTGCT CTCCAATTTG GGTTCCACCC CAGTCTTAAT
GACTACAAGG TTGTAAGGAT GATGCGTACC AACAAAGGTG CCTTGGCGGT TGAGGTTTAT
ACTCTCAGAA CGGACTCTTG GAAGATGATT GAAGCAATTC CTCCTTGGTT AAAATGCACT
TGGCAGCATC ATAAGGGTAT GTTTTTTAAA GGAGTAGCAT ACAGCATCAT TGAGAAAGGT
CCTATGTTCA GCATTATGTC ATTTGATTCA GGCAGTGAAG AATTCGAAGA ATTCATAGCA
CCAGATTCCA TTTGCAGTCC ATCTGAGTTA TGTATTGACG TTTACAAGGA ACAAATTTGC
TTGCTTTTTA GCTTTTACTC TTGTGAGGAG GAGGGCATGG TACCAAATGA CTTATGGGTT
CTGCAAGAAA AACAGTGGAA ACAATTGCGT CCTTTTATTT ATCCTGCCGG TAGTTATGGT
ACAATCGGGA TTAGTATAGA TAATGAACTC TTAAAGGTAA GAAGAGATTT CCTTAGGGGC
GTAGGAAATC TGTGTTTGTG TAATTACGAA TCCAAGCAAC TTCTTGAAAC GGGAATTGAG
TTGGCCATCA TCACATACGG CGAAATCGAA TTCTTG

EF053403:SFB9 gene, partial cds., 1061 bp

TTCTCTTGTT CGGTTTATTT GCACATGCAA GTCTTGGAGT GATTTGATTG GCAGCTCGAG
TTTTGTTAGC ACACACCTTC ATAGGAATGT CACAAAACAT GCCCATGTCT ATTTACTCTG

CCTCCACCAC CCGAATGTTG AACGCCAGGC TGATCCTGAC GACCCGTATG TTGAACAAGA
ATTTCAATGG TCTCTATTTT CCAATGAAAC ATTTGAGGAT TGCTCCAAGT TAAGCCATCC
CTTGGGGAGC ACAAAACATT ATGTGATATA TGGCTCAAGC AATGGTTTAG TTTGCATTTC
GGATGAGATG CTGAATTTTG ATAGTCCTAT ACACATATGG AACCCATCGG TTAGGAAACT
TAGAACCGCT CCAATCAGCA GCAACATTAA CATTAAATTT AGCCATGTTG CTCTCCAATC
CGGGTTCCAC CCCGGGGTTA ATGACTACAA GGCTGTCAGG CTGATGCGCA CCAACAAACG
TGCCTTGGCA GTTGAGGTTT ATAGTCTCAG AACAGACTCT TGGAAGATGA TTGAAGCAAT
TCCTCCTTGG TTAAAGTGCA CTTGGCAGCA TTATAAGGGT ACATTTTTTA ATGGAGTAGC
ATACCACATC GTTGAGAAAG GTCCTATATT CAGCATTATG TCCTTTGATT TAGGCAGTGA
ACAATTCGAA GAATTCATAG CACCAGATGC CATTTGCAGT TCATGGGGGT TATGTATTGA
CGTTTACAAG GGACAAATTT GCTTGCTTTC AAAATGTTAT GGTTGTGAGG AGGAGGGCAT
GGAGAAAATT GACTTATGGG TTCTGCAAGA AAAACTGTGG AAACAATTGT TTCCTTTTAT
TTATCCTTTT GGTTATTGTT ATGATATAAT CGGTATTAAT ATAGATGATG AACTGTTAAT
GGGAAGAACA GATATAGCTA AGGGCGTAGC AGATCTATTT TTGTGTAATT ACGAATCCAA
GCAAGTTCGT GAAACAGGAA TCAAGTTGGG CCTGATGTCA TATGGGGAAA TCGAATCCTT
GTGTTCAATT ACTTACATAG AAAGCATGGT TTTACTCAAT A

EU652884: SFB11 gene, complete cds. 1134 bp

ATGACATTCA GGCTACGGAA GAAAGAAATC TTAATCGACA TCCTGGTGAG ACTACCTGCA
AAATCCCTAG TTCGGTTTCT GTGCACATGC AAGTCATGGA GTGATTTGAT TGGCAGCTCC
TGTTTTGTTA GCTCACAACT TCATAGGAAT GCCACAAAGC ATGACCATGT CTATCTACTT
TGCCTCCACC ACTCAAATTT TGAACTTCAG GCTGACCCTG ATGACCCATA TGTCAAACAA
GAATTTCAAT GGTCTCTTTT TTCCAATGAA ACATTTGAGG AGTGCTCTAA GTTAAGCCAT
CCCTTAGGGA GCACAGAACA TTATGTTATA TATGGCTCAA GCAATGGTTT AGTTTGCATT
TCGGATGAGA TATTGAATTT TGATAGTCCT ATACACATAT GGAACCCATC GGTTAGGAAA
ATTAGAACCA CTCCAATCAG CACCAACATT AACATTAAAT TTAGCCATAT TGCTCTGCAA
TTCGGGTTCC ACCCCGGAGT TAATGACTAC AAAACTGTGA GGATGATGCG CACCAACAAA
GATGTCTTGG CAGTTGAGGT TTATAGTCTT AGAACAGACT CTTGGAAGAT GATTGAAGCA
ATTCCTCCCT GGTTAAAATG CACTTGGCAG CATCATAAGG GTACATTTTT TAATGGAGTT
GCATACCACA TTATTATTAA GAAAGGTCCT ATATTCAGCA TTATGTCATT CGATTCAGGC
AGTGAAGAAT GCGAAGAGTT CATAGCACCA GATGCCATTT GCAGTCCATG GGGGTTATGT
ATCGACGTTT ACAAGGAACA AATTTGCTTG CTTTTTAGAT GTTATGGGTG TGAGGAGGAG
GGCATGGACA AAGTTGACTT ATGGGTTCTG CAAGAAAATC GATGGCAACA AACGTATCCT
TTTATTTTTC CTTTCAATTA TTGTGATCGT ATAGTCGGAA TTAGTATGGA TAATGGACTC
TTGATGGAAA AAAGAGATTT TGGAAAGGGC GCAGTAGATC TGTATTTGTG TAACTACGAA
TCCAAGCAGG TTCTTGAAAC AAGAATTAAG TTGGCTGTCA TGAAATATGA CGAAATCGAA
TTCTTGTTTG CAATTACTTA CAGGGAAAGT TTGGTTTTAC TCAACAATTA TTAA

EF062342: SFB-13 gene, partial cds. 693 bp

AACATCCTAG TAAGACTACC TGCAAAATCC CTTGTTCGGT TTCTGTGTAC GTGCAAGTCA
TGGAGTGATT TGATTGGGAG CTCCTGTTTT GTCAGCACAC ACCTTCATAG GAATGTCACA
AAACATACCC ATGTCTATCT ACTATGCCTC CACCACCCAA ATTTTGAACG AAACGACGAC
CCTGATGACC CATATGTTGA ACAAGAATTT CAATGGTCTC TTTTTTCCAA TGAAACATTT
GAGGAGTGCT CCAAGTTAAG ACATCCCTCA GGGAGCACAG AACATTATAT GATATATGGC
TCAAGCAATG GCTTAGTTTG CATTTCGGAG GAGATATTGA ATTTCGATAG TCCAATACAC
ATATGGAACC CCTCGGTTAA GAAATTTAGG ACCCCTCCAA TGAGCACCAA CATTAACATT
AAATTTAGCT ATGTTGCTCT CCAATTCGGG TTCCACCCCG GGGTTAACGA CTACAAGGCT
GTAAGGATGA TGCGTACCAA CAAAAATGCC TTGGCGGTTG AGGTTTATAG TCTCAAAACA
GACTCTTGGA AGATGATTGA AGCAATTCCC CCGTGGTTAA AATGTACTTG GCAGCATCAT
AAGGGTACAT TTTTTAATGG AGTAGCATAC CACGTCCTTC TGAAAGGTCC TATATTCAGC
ATTATGTCAT TCGATTCAGG CAGTGAAGAA ATA

DQ897929:SFB14 gene, partial cds., 1250 bp

TTAGCTATTS TMACCTCAAA AGTGAGTCGT TGGACTTTCT GCAAAAATGA AAAGCACTAT
TGTTTTCCTT GTTTTGTTAT GATCAATATT TTGGATGCTA ACTTATTTTG ATTATGATTT
CTTCAGGATG ACATTCACAC TACGTAAGAA AGAAATCTTA ATCGACATCC TAGTAAGACT
ACCTGCAAAA TCCCTTATTC GCTTTCTGAG TACATGCAAG TCGTGGAGTG ATTTGATTGG
AAGCTCAATT TTTGTTAGCA CACACCTTTG TAGGAATGTG ACAAAACATA CCCATGTTTA
TCTACTTTGC CTCCACCACC CAAATTTTGA GCGTTTGGTC GACCCTAATG ACCCATATCT
TAAAAAGGAA TTTCAATGGT CTCTTTTTCC CAAAGAAACA TTTGAGGAGT GCTACAAACT

AAGCCATCCC TTAGGGATGA CAGAACATTA TGGGATATAT GGTTCAAGCA ATGGTTTAGT
TTGCATTTCG GATGAGATCC TGAATTTCGA TAGTCCTATA CACATATGGA ACCCATCGGT
TAGGAAATTT AGGGCCCTTC CAATGAGCAC CAACATTAAC ATTAAATTTA GCTGTGTTGC
TCTCCAGTTC GGGTTCCACC CTGGGGTTAA TGACTACAAG GCTGTAAGGA TGATGCGTAC
CAATAAAAGT GCCTTGGCGG TTGAGGTTTA TAGTCTCAAA AGAGACTCTT GGAAGATGAT
TGAAGCAATT CCTCCTTGGT TAAAATGCAC TTTGGAACAT CATAGGGGTA CGTTTTTCAG
TGGAGTAGCA TACCACATCA TTCAGAAAGG TCCTATGCTC AGCATTATGT CATTCGATTC
AGGCAGTGAA AAATTCGAAG AAATCATAGC ACCAGATGCC ATTTGCAGTT TATGGGGGTT
ATATATTGAC GTTTACAAGG AACAAATTTG CTTGCTTTTT ATATGTTATG GGTGTGAGGA
GGAGGGCATG GAAAAAGCTG ACTTATGGGT TCTGCAAGAA AAACGGTGGA AACAATTGAG
TCCTTTTATT TATCCTCCGG ATTATTATTA TTGTACAACA GGGATTAGTA TGGATAACAA
AATCTTAATG CTAAGAGTAG ATGACATTAG AGGCATAAGA AATCTGGATT TATGTGATTA
CGAATCCAAC CAAGTTCTTG AAACAGGAAT TGAGTTGGCC ACCATGAAAT ATGGCGAAAT
CGAATTCTTG TTTTCAATTA CTTACGTAGA AACTTTACCT TTACTCAATT

DQ887488:SFB14', complete cds 1609 bp

TTGGCTATTR TCAACCGCAA AAGTGAGTCG TTGGACTTTC TGCAAAAATG AAAAGCACTA
TTGTTTTCCT TGTTTTGTTA TGATCAATAT TTTGGATGCT AACTTATTTT GATTATGATT
TCTTCAGGAT GACATTCACA CTACGTAAGA AAGAAATCTT AATCGACATC CTAGTAAGAC
TACCTGCAAA ATCCCTTATT CGCTTTCTGA GTACATGCAA GTCGTGGAGT GATTTGATTG
GAAGCTCAAT TTTTGTTAGC ACACACCTTT GTAGGAATGT GACAAAACAT ACCCATGTTT
ATCTACTTTG CCTCCACCAC CCAAATTTTG AGCGTTTGGT CGACCCTAAT GACCCATATC
TTAAAAAGGA ATTTCAATGG TCTCTTTTTC CCAAAGAAAC ATTTGAGGAG TGCTACAAAC
TAAGCCATCC CTTAGGGATG ACAGAACATT ATGGGATATA TGGTTCAAGC AATGGTTTAG
TTTGCATTTC GGATGAGATC CTGAATTTCG ATAGTCCTAT ACACATATGG AACCCATCGG
TTAGGAAATT TAGGGCCCTT CCAATGAGCA CCAACATTAA CATTAAATTT AGCTGTGTTG
CTCTCCAGTT CGGGTTCCAC CCTGGGGTTA ATGACTACAA GGCTGTAAGG ATGATGCGTA
CCAATAAAAG TGCCTTGGCG GTTGAGGTTT ATAGTCTCAA AAGAGACTCT TGGAAGATGA
TTGAAGCAAT TCCTCCTTGG TTAAAATGCA CTTTGGAACA TCATAGGGGT ACGTTTTTCA
GTGGAGTAGC ATACCACATC ATTCAGAAAG GTCCTATGCT CAGCATTATG TCATTCGATT
CAGGCAGTGA AAAATTCGAA GAAATCATAG CACCAGATGC CATTTGCAGT TTATGGGGGT
TATATATTGA CGTTTACAAG GAACAAATTT GCTTGCTTTT TATATGTTAT GGGTGTGAGG
AGGAGGGCAT GGAAAAAGCT GACTTATGGG TTCTGCAAGA AAAACGGTGG AAACAATTGA
GTCCTTTTAT TTAGAGATAT TTAGTGATAT ACCCATTCTT AACACTAATA TTATAGATAA
ACCCTCCATC ATTGAATTTC TATAAACAAA CCCAAAAAAA ACCCAAAAAA TGACAACTGG
CCTTATTGAA TTTAATATTG ATTATTAAAT TACTTTAATG CCCTATTGAG TGTTTTGGGT
ATTTTTATGA GATTTTGGGG TTGGGCTTGT TTTAAGAAAT TTATGGTAGT TTTGTAATTT
CTAAGAAGTT AAAGCCTTTT TGTTATATTG TAAATGGGTT TTGGGTGTGT TTATAAAGTT
CATTTCATAT AGGGTATTTT TATAATTTGG GTCTTCATAT TGGGTATATT AGTAAATCTC
CCTTTTATTT ATCCTCCGGA TTATTATTAT TGTACAACAG GGATTAGTAT GGATAACAAA
ATCTTAATGC TAAGAGTAGA TGACATTAGA GGCATAAGAA ATCTGGATTT ATGTGATTAC
GAATCCAACC AAGTTCTTGA AACAGGAATT GAGTTGGCCA CCATGAAATA TGGCGAAATC
GAATTCTTGT TTTCAATTAC TTACGTAGAA ACTTTACSCT TACTCAATA

EU652885: SFB17 gene, complete cds 1131 bp

ATGGCATCGA CACTACGTAA GAAAGAAATC TTAGTCGACA TCCTACTAAG ACTACCTGCA
AAATCCCTCG TTCGGTTTCT TTGTACATGC AAGTTATGGA GTAATTTGAT TTGCAGCTTG
AGTTTCGCTA GCACACACCT TCACAGGAAT GTCACAGGAC ATGCCCATGC CTATCTACTT
TGCCTCCACC AGCCAAATTT TGAATGTCAA AGGGACGATG ATGACCCATA TTTTAAAGAA
GAACTCCAAT GGTCATTGTT TTCCAATGTA ACATTTGAGC AGTGCTGCAC GTTAAGCCAT
CCATTAGGGA GCACAGAACA TTATGGGATA TATGGTTCAA GCAATGGTTT AGTTTGCATT
TCGGATGAGA TATTGAATTT TGATAGTCCT ATACACATAT GGAACCCATC GGTTAGGAAA
CTTAGGACCC CTCCAATGAG CACCAACACT AACATTAAAT TTAGCTATGT TGCTCTTCAA
TTCGGGTTTC ACTCCGGAGT TAATGACTAC AAGGTTGTTA GGATGATGCG TACCAACAAA
AATGCCTTGG CGGTTGAGGT TTATAGTCTT GGTACAGACT GCTGGAAGCT GATTCAAGCA
ATTCCTCCTT GGTTAAAATG CACTTGGAAG CATCACAAGG GTACATTTTT GAATGGAGTA
GCATACCACA TCATTGAGAA AGGTCCTATA TTCAGCATTA TGTCCTTCGA TTCAGGCAGT
GAAGACTTCG AAGAATTCAT AGCACCAGAT GCCATTTGCA ATTCATGGAA GTTATGCATC
CAAGTTTACA AGGAACAGAT TTGCTTGCTT TTTGGATTTT ATGGTTGTGA GGAGGAGGGC
ATGGAAAACA TTGACATATG GGTTCTGCAA GAAAAACGGT GGAAACAATT GTATCCTTTT
ATTTATGATC CTTTGGATTG CTGTTATCAG ATAATCGGGA CTAGTATAAA CAATGAACTC
TTAATGGCAA GAAGAGATTT CGATGACGGG GTAGTAGGTC TGCAATTGGG TAATTACGAA
TCCAAGCAAG TTCTTGACAC AGGGATTAAG TTGGCCATCA TGAGATATGG GGAAATCGAA
TTCTTGTTTG CAATTACTTA CATAGAAAGT TTAGTTTTAC TCAATAACTA A

FJ377726: SFB22 gene, partial cds. 448 bp

ATGACATTCA CACTACGTAA GAAAGAAATC TTAATCGACA TCCTACTAAG ACTACCTGCA
AAATCCCTCA TTCGGTTTCT AAGTACATGC AAGTCATGGA GTGATTTGAT TGGCAGCTCG
AGTTTTGTTA GCACACATCT TAATAAGAAT GTCACAAAAC ATGCCCATGT CTATCTACTT
TGCCTCCACC ACCCAAATTT TGAATGTGTG ATCGACCCCG ATGACCCATA TTTAGAAGAA
GAACTTCAAT GGTCTCTGTT TTCCAATGTA ACATTTGAGA AGTGCTCCAA GTTAAGCCAT
CCCCTAGGAA GCACAAAACA TTATGGGATA TATGGTTCAA GCAATGGTTT GCTTTGCATT
TCTGATGAGA TATTGAATTT CGATAGTCCA ATACACATAT GGAACCCATT GGTTGGAAGA
TATAGGACCA CTTCAATGAG CACCAACA

EU652886: SFB23 gene, partial cds. 1083 bp

ATGATATTCA GACTACGTAA GAAAGGACTT TTAATCGACA TCCTAGTAAG ACTACCTGCA
AAATCCCTTG TTCGTTTTCT GTGTACATGT AAGTCTTGGA GTGATTTGAT TAGCAGCTCG
AGTTTTGTTA GCACACACCT TAACAGGAAT GTCGCAAAAC ATGAACATGT CTATCTCCTC
TGCCTCCGCC ACCCAAATGT TAGACGTCAG GTTGACCGTG ATGACCCGTA TGTTAAAAAA
GAATTTCAAT GGTCTCTTTT TTCCAATGAA ACATTTGAAG AGTGCTCCAA GTTAAGCCAT
CCCTTAGGGA GCACAGAACA TTATGGAATA TATGGTTCAA GCAATGGTTT AGTTTGCATT
TCGGATGTGA TATTGAATTT CGATAGTCCT ATACACATCT GGAACCCATC GGTGAGGAAA
TTTAGGACCC CTCCAATGAG CACCCACATT AACATTAAAT TTACCTATGT TGCTCTCCAA
TTCGGGTTCC ACCCTGGGGT TAATGACTAC AAGACTCTGA GGATGATGCG TACCAACAAA
GGTGCCGTGG GAGTTGAGGT TTATAGTCTT AGAACAGACT CTTGGAAGAT GATTGAAGCA
ATTCCTCCTT GGTTAAAATG TACTTGGCAG CATCACAGGG GTACGTTTTT TAATGGAGTA
GCATACCACA TCATTCAGAA AGGTCCTATA CTCAGCATTA TGTCGTTCGA TTCAGGCAGT
GAAGGATTCG AAGAATTCAT AGCACCAGAT GCCATTTGCA GTCAATGGGG GTTATGTATT
GATGTTTACA AGGAACAAAT TTGCTTGCTT CTTAAGTTTT ATTCTTGTGA GGACGAGGGC
ATGAGGAAAA TTGACGTATG GGTTCTGCAA GAAAAACGTT GGAAACAATC GTGTCCTATT
ATTTTTCCTT CGGATTATAA TTATCGTACT ATCGGGATTA CTATAGATAC TAAATTCTTA
ATGCTAAGAA CGGATTACGA TAAGGGCATA GCAAATCTGC ATTTGTGTGA TTACGAATTC
AAGCAGGTTC TTGAGACGGG AATTAAGTTG GCAGTCATGA AATATGACGA AATCGAATTC
GTG

EU836685: SFB24 gene, partial cds 1080 bp

ATGACATTCA CTCTATGTAA GAAAGAAATT TTAGTCGACA TCCTGGTAAG ACTGCCTGCA
AAATCTCTCG TTCGGTTTAT GTGTACATGT AAGTCATGGA GTGATTTGAT TGGCAGCTCC
AGTTTTGTTA GCACACACCT TAATAGGAAT GTCAATAAAC ATGCCCATGT ATATCTACTT
TGCCTCCACC ATCCAAATTT TGAATGTGTG GTGGACCCTG ATGACCCATA TTTAGAAGAA
GAAGTTCAAT GGTCTCTTTT TCCCAATGAA ACATTTGAGG AATGCTCCAA GTTAAACCAT
CCCTTAGGGA GCACAGAACA TTATGGGATA TATGGCTCCA GCAATGGTTT AGTTTGCATT
TCGGATGAGA TATTGAATTT CGATAGTCCT ATACATATAT GGAACCCATC AGTTAGGAAA
CTTCGGACCC TTCCAATAAG CACCAACATC ATTAAATTTA GCCATGTTGC TCTCCAGTTC
GGGTTCCATC CCGGGGTTAA TGACTATAAG GCTGTAAGGA TGATGCGTAC CAACAAAAAT
GCCTTGGCGG TTGAGGTGTA TAGTCTTAGA ACAGACTCTT GGAAGATGAT TGAAGCAATT
CCTCCTTGGT TAAAGTGCGC TTGGCAGCAT TATAAGGGTA CATTTTTCAA TGGAGTAGCA
TACCATATCA TTGAAAAAGG TCCTATATTC AGCATCATGT CCTTCGATTC AGGCAGTGAA
GAATTTGAGG AATTCATAGC ACCAGATGCA ATTTGCCGTC CATCTGAGGT ATGTATTGAC
GTTTACAAGG ACCAAATTTG CTTGCTTTTT GGATTTTATC GTTGTGAGGA GATGGGCATG
GATAAAATTG ACTTGCGGGT TCTGCAAGAA AAACGGTGGA AACAGTTGTG TCCTTTTATT
TATCCATGGG GTTTTTGTTA TCGTATAACC GGGATTAGTA TAGATAATGA ACTCTTAATG
GGAAGAAGAG ATTTCTTAAA GGGCGTAGCA GATCTCTATT TGTGTAAGTA CGAATCCAAG
CAGGTTCTTG AAACAGGAAT TAAGTTGGGC ATCATGAAAT ATGGCGAAAT CGAATTCGTG

EU836686: SFB25 gene , partial cds. 1083 bp

ATGGCATTCA TACAACGTAA GAAAGAGATC TTAATCGACA TCCTAGTGAG GTTACCAGCA
AAATCACTCG TTCGATTTCT GTGTACATGC AAGTCATGGA GTGATTCGAT TGGCAGCTCG
AGTTTTGTTA GCACACACCT TCGTAGGAAC GTGACAAAGC ATTCCCATGT CTATCTACTT
TGCCTCCACC ACCCAAATTT TGAATGTGCG GTCGATCCTA ATGACCCATA TATAGAAGAA
GAAGTTCAAT GGTCTCTTTT TTCCAATGAA ACATTTGAGC AGTGCTCCAA GTTAAGCCAT
CCCTTAGGGA GCACGGAACA TTATGTGATA TATGGTTCAA GCAATGGTTT AGTTTGCATT
TCTGATGAGA TATTGAATTT TGATAGTCCT ATACACATAT GGAACCCATC GGTTAGGAAA

CTTAGGACCC CTCCAATCAG CACCAACATT AACATTAAAT TTAGCTGTGT TGCTCTCCAA
TTCGGGTTCC ACCCTGGTGT TAATGATTAC AAGGCTGTGA GGATGATGCG TACCAACAAA
GGTGCTTTAG CAGTTGAGGT TTATAGTCTT AAAACAGACT GTTGGAAGAT GATTGAAGCA
ATTCCTCCTT GGTTAAAATG CACTTGGCAG CACCACGATC GTACATTTTT CAATGGAGTA
GCGTACCACA TCATTGAGAA AGGTCCTATA TTCAGCATTA TGTCCTTCGA TTCAGGCAGT
GAAGAATTTG AAGAATTCAT AGCACCAGAT GCCATTTGCA GTCCATATGA GGCATGTATT
GACGTTTACA AGGAACAAAT TTGCTTGCTT TTTGAATTTT ATGATTGTGA GGAGGAAGGC
ATGGACAAAA TTGACTTCTG GGTTCTGCAA GAAAAACGGT GGAAACAATT GTGTCCTTTT
AGTTATCCTT TGGGTTATTG TTATCGTATA ACCGGGATTA GTATAGACAA TGAACTCTTA
ATGGGAAGAA GAGATTTCGC TAAGGGCGGA GCAGAACTGT ATATATGTAA TTACGAATCC
AAGCAAGTTC TTGAAACAGG AATTAAGTTG GGCAGCATGA AATATGGCGA AATCGAATTC

EU652887: SFB26 gene, complete cds. 1128 bp

ATGACATTCA CACTACGTAA GAAAGAAATC TTAATCGACA TCCTAATAAG ACTACCTGCA
AAATCCCTTG TTCGGTTTCT GTGTACATGC AAGTCATGGA GTGATTTGAT TGGCAGCTCC
AGTTTTGTCG GCACACACCT TCATAGGAAT GCCACAAAAC ATACCCATGT CTACCTACTA
TGCCTCCACC ACCCAAATTT TGAACGAAAC GATGACCCTG ATGACCCATA TGTTGAACAA
GAATTTCAAT GGTCTCTTTT TTCCAATGAA ACATTTGAGG AGTGCTCCAA GTTAAGCCAT
CCCTCAGGGA GCACAGAACA TTACGTGATA TATGGCTCAA GCAATGGCTT AGTTTGCATT
TCGGAGGAGA TATTGAATTT CGATAGTCCA ATACACATAT GGAACCCCTC GGTTAAGAAA
TTTAGGACCC CTCCAATGAG CACCAACATT AACATTAAAT TTAGCCATGT TGCTCTCCAA
TTCGGGTTCC ACCCCGGGGT TAACGACTAT AAGGCTGTAA GGATGATGCG TACCAACAAA
AATGCCTTGG CGGTTGAGGT TTATAGTCTC AGAACAGACT CTTGGAAGAT GATTGAAGCA
ATTCCCCCTT GGTTAAAATG CACTTGGCAG CATCATAAGG GTACATTTTT TAATGGAGTA
GCATATCATA TCATTGAGAA AGGTCCTATA TGCAGCATTA TGTCATTCGA CTCAGGCAGT
GGAGAATTCG AAGAATTCAT AGCACCGGAT GCCATTTGCA GTCCATCTGA GTTATGTATT
GATGTTTACA AGGAACGAGT TTGCTTGCTT TTTAGCTTTT ATTCTTGTGA CGAAGAGGGC
ATGGTACCAA ATGACTTATG GGTTCTGCAA GAAAAACGAT GGAAACAATT GTGTCCTTTT
ATTTATCCTG CGGGTAGTTA TGGTACAATC GGGATAACTA TAGATAATAA ACTCTTAATG
GTAAGAAGGG ATTTCCTTAG GGGCGCAGGA GATCTGTGTT TGTGTAATTA CGAGTCCAAG
CAAGTTTTTG AAACAGGAAT TGAGTTGGCC GTCATGAAAT ATGGCGAAAT CGAATTCTTG
TTTGCAATTA CTTACATAGA AAGTTTGGCT TTACTTAATA AATATTGA

EU836687: SFB27 gene, partial cds. 1059 bp

GAAATCTTAA TCGACATCCT CGTAAGACTA CCTGCAAAAT CCCTTGTTCG GTTTCTGTGT
ACATGCAAGT CGTGGAGTGA TTTGATTGGC AGCTCGGGTT TTGTTAGTAC ACACCTTCAT
AGGAATGTCA CAAAACATGC CCATGTCTAT CTACTTTGCC TCCACCACCC AAACTTTGAA
CGTCAGAACG ACAATGATGA CCCATATGAT ATAGAAGAAC TTCAATGGTC ACTTTTTTCC
AATGGAACAT TTGTGCAGTT CTCCAATTTA AGCCATCCTT CAGAAAACAC AGAGCATTAT
AGGATATATG GTTCAAGCAA TGGTTTAGTT TGCATTTCGG ATGAGATATT GAATTTCGAT
AGTCCTATAC ACATATGGAA CCCATCGGTC AGGAAATTTA GGACCACTCC AACGAGCACC
AACATTAACA TTAAATTTAG CTATGTTGCT CTCCAATTTG GATTCCACCC CGGGGTTGAT
GACTACAAGG CTGTAAGGAT GATGCGTACC AACAAAAATG CCTTGGCGGT TGAGGTTTAT
AGTCTCAGAA CAGACTCTTG GAAGATGATT GAAGCAATTC CTCCTTGGTT AAAATGCACT
TGGAAAAATC ATAAGGATAC ATTTTTTAAT GGAGTAGCAT ATCACATAAT TGAGAAAGGT
CCCATCTTCA GCATTATGTC CTTTGATTCA GGCAGTGAAG AATTCGAAGA ATTCATAGCA
CCAGATGCCA TTTGCGGTCC ATGGGGTTTA TGTATCGACA TTTACAAGGG ACAAATTTGC
TTGCTTTGTA GATATTATGG TTGTGAGGAG GATGGCATGG AAAAAGTCGA CTTATGGGTT
CTGCAAGAAA AACGGTGGAA GCAATTGTGT CCTTTTATTT TTCCTTGGGA TGAATGGAAC
GTTACAATCG GGATTAGCAT AGATGATGAA CTGTTAATGG AAATAAGAGA TTTCGATAAG
GGCGTAGCAG ATCTGTATTT GTGTAATTAC GAATCCAAGG AAGTTCTTAA AACAGGAATT
AAGTTGGCCG TCATGAAATA TGGCGAAATC GAATTCGTG

FJ377729: SFB37 gene, partial cds. 470 bp

TATTATTTTC TATCAGGATG ACATTCAGAC TACGTAAGAA AGAAATCTTA ATCGACATCC
TAGTAAGACT ACCTGCAAAA TCCCTTGTTC GGTTTCTGTG TACATGCAAG TCGTGGAGTG
ATTTGATTGG CAGCTCGGGT TTTGTTAGTA CACACCTTCA TAGGAATGTC ACAAAACATG
CCCATGTCTA TCTACTTTGC CTCCACCACC CAAACTTTGA ACGTCAGAAC GACAATGATG
ACCCATATGA TATAGAAGAA CTTCAATGGT CACTTTTTTC CAATGGAACA TTTGTGCAGT
TCTCCAATTT AAGCCATCCT TCAGAAAACA CAGAGCATTA TAGGATATAT GGTTCAAGCA
ATGGTTTAGT TTGCATTTCG GATGAGATAT TGAATTTCGA TAGTCCTATA CACATATGGA
ACCCATCGGT CAGGAAATTT AGGACCACTT CAATGAGCCA CCAACAAAAA

- ***SFBs* sequences identified in this work**

Sequences: 35
Minimum Sequence Length: 594
Maximum Sequence Length: 764
Average Length: 693
Primer: SFBc(F+R)

>Altera-E_705bp:_SFB2

Primer SFBc(F)▶▶▶▶▶▶▶
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TTAACAGCCCGAGTTTTATCACCACACAGCTTAACAGGAATGTCACCAAACACCTCCATGTCTCTCTACTTTGCC
TCCACTACCCCGATCTCAAACGTCCTTTCGAGTTCTACGAAAACTACGAGGACTACCCAGATCTTAAACGAGAAT
TGGAATGGTCACTTTTCTCCAACGAAACATTTGAGCATTGCTCCAAGTTAAACCATCCCTTGGGGATCAAGAAAG
ATTATAGGGTATATGGCTCAAGCAATGGCCTAGTTTGCATTTCGGATGACAAATTGGACACCAAGAGCCCTATAC
ACATATGGAACCCCTCGGTTAGGAAATTTAGAACCCTTCCAATGAGCACCAACGTTAAATTTCGCTATATTGCTC
TCCAATTCGGGTTCCACCCCGGGGTTAACGACTACAAGGTTGTAAGGATGTTGCGCGTCCACAAAGATGATGCTT
TCGCAGTCGAAGTTTATAGTCTCAGCACAGACTCTTGGAAGATGGTTGAAGAACATCCTCTTTGGTTAAAATGCA
CTTGGCAGACCACAGGGGTACATTTTATAATGGAGTAGCATACCACATTATTGAGAAATTTCCTCTATTCAGCGT
TATGTCATTCGATTCAGGCAGTGAAGAAAT
◀◀◀◀◀ Primer_SFBc(R)

>Altera-F_696bp:_SFBC

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TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAACCATACCCATGTTTATCTACTTTGCC
TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATTATGGGATATATG
GCTCCAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCAATTCCTCCCTGGTTAAAATGCACTTTGCAACATC
ATAGGGGTACGTTTTTCAGTGGAGTACCATACCACATCATTTCCCAATAGGTCCTACGCTCCGCACTATGTCCTC
TCATTTCGGCATTGAATAACA

>BO92639095-B_694bp:_SFB1

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TCCACCACCCAAATTTTGAACGTCTGGCCGACCCTGATGATCCATATGTTAAGCAAGGATTTCAGTGGTCTCTTT
TTTCTAATGAAACATTTGAGGAGTGCTCCAAGTTAAGCCATCCCTTAGGGAGCAGAGAACCTTATGTGATTTATG
GCTCAAGCAATGGTTTAGTTTGCATTTCCGATGAGATACTGAATTTCGATAGTCCTATACACATATGGAATCCAT
CGGTTAGTAAACTTAGAACCACGCCAATCAGCACCAATATCAGCATTAAATTTAGCCATGTTGCTCTCCAATTCG
GGTTCCACCCCAGGGTTAATGACTTCAAGGCTATAAGGATGTTGCGTACCAACAAAAAGGCATTGGCGGTTGAGG
TTTATAGTCTCAGAGCAGACTCCTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
ATGACGGCACATTCTTTAATGGAGTAGCATACCACATCATTGAAAAAGGTCCTATATTCAGTATTATATCCTTCG
ATTCAGGCAGTGAAGAAAT

>BO93622312-F_695bp:_SFB1

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TTGGCAGCTCGAGTTTTGTTAGCATACACCTTAACAGGAATGTCACAGAACATGCCCATGTCTATCTACTCTGCC
TCCTCCACCCAAATTTTGAACGTCTGGCCGACCCTGATGACCCATATGTTAAACAAGAGTTTAGCTGGTCTCTTT
TTTCGAATGAAACATTTGAGGAAAGCTCCAAGATAACCCATCCCTTAGGGAGCACAGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCAGATGAGATTTTGAATTTCGATAGTCCTATACACATATGGAACCCGT
CGGTTAAGAAATTTAAGACCCCTCCAATGAGCACCAACATTAACATTAAATTTAGCCTCGTTTCTCTCCAATTCG
GGTTCCATCCTAGGGTTAATGACTACAAGGCTGTAAGGATGATGCGCACCAACAAAAATGTCTTGGCGGTTGAGG
TTTATAGTCTCAGCACAGACTCTTGGAAAATGGTTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC

ATAACGGGTACATTTTTTAGTGGAGTAGCATACCGCATCATTCAGAAAGGTCCTCTATTCAGCATTATGTCCTTC
GATTCAGGCAGTGAAGAAAT

>BORA-A 694bp: SFBC

TCGACATCCTAGTAAGACTACCTGCAAAATCCCTTATTCGCTTTCTGAGTACATGCAAGTCGTGGAGTGATTTGA
TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTTGCC
TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAGAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTACTACAAACTAAGCCATCCCTTAGGGACGACAGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTTGGAACATC
ATAGGGGTACGTTTTTCAGTGGAGTAGCATACCACATCATTCAGAAAGGTCCTATGCTCAGCATTATGTCATTCG
ATTCAGGCAGTGAAGAAAT

>BORA-B 695bp: SFB9

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TTAGCAGCCCGAGTTTTGTCAGCACACACCTTTATAGGAATGTCACAAAACATGCCCATGTCTATCTACTTTGCC
TTCACCACCCGAATTTTGAATGTGTGGTCGACCCTGATGACCCATATTTGGAAGAAGAGCTTCAATGGTCTCTTT
TTTCCAATGAAACATTTAAGCTGTGCTCCAAGTTAAGCCATCCCTTAGGGAGTACAAATCGTTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATACTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTAAAGAAATTTACGAGCCCTCCAATGAGCACCAACATTAACGTCAAATTTGCCTATGTTGCTCTCCAATTTG
GGTTCCACCCCAGTCTTAATGACTACAAGGTTGTAAGGATGATGCGTACCAACAAAGGTGCCTTGGCGGTTGAGG
TTTATACTCTCAGAACGGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
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GATTCAGGCAGTGAAGAAAT

>CORNIA-B 694bp: SFBC

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TTGGCAGCTCGAGTTTTGTTAGCATACACCTTAACAGGAATGTCACAGAACATGCCCATGTCTATCTACTCTGCC
TCCTCCACCCAAATTTTGAACGTCTGGCCGACCCTGATGACCCATATGTTAAACAAGAGTTTAGCTGGTCTCTTT
TTTCGAATGAAACATTTGAGGAAAGCTCCAAGATAACCCATCCCTTAGGGAGCACAGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCAGGTGAGATTTTGAATTTCGATAGTCCTATACACATATGGAACCCGT
CGGTTAAGAAATTTAAGACCCCTCCAATGAGCACCAACATTAACATTAAATTTAGCCTCGTTTCTCTCCAATTCG
GGTTCCATCCTAGGGTTAATGACTACAAGGCTGTAAGGATGATGCGCACCCAACAAAAATGTCTTGGCGGTTGAG
TTTATAGTCTCAGCACAGACTCTTGGCAAAATGGTTTGAAGCAATTCCTCCTTGGTTAAAATGCACTTCGCAGCA
TCATCACGGGTACATTTCTTTACTGCAGTAGCATACCACATCATTCAGAACGGTCTCTATTCATCATATGTCTCG
ATTCAGGCAGTGAAGAAAT

>CORNIA-C 765bp: SFB1

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TTGGCAGCTCGAGTTTTGTTAGCGTACACCTTAACAGGAACGTCAAAAAGCACGCCAATGTTTATCTACTCTGCC
TCCACCACCCAAATTTTGAACGTCTGGCCGACCCTGATGATCCATATGTTAAGCAAGGATTTCAGTGGTCTCTTT
TTTCTAATGAAACATTTGAGGAGTGCTCCAAGTTAAGCCATCCCTTAGGGAGCAGAGAACCTTATGTGATTTATG
GCTCAAGCAATGGTTTAGTTTGCATTTCCGATGAGATACTGAATTTCGATAGTCCTATACACATATGGAATCCAT
CGGTTAGTAAACTTAGAACCACGCCAATCAGCACCAATATCAGCATTAAATTTAGCCATGTTGCTCTCCAATTCG
GGTTCCACCCCCAGGGTTAATGACTTCAAGGCTATAAGGATGTTGCGTACCCAACAAAAAGGCATGGGCGGTTGA
GGTTTATAGTCTCACACCAAACTCCTGCAATATGATCGCGCCACTCCTCCCTGGCCTACACGCCACTTGCCCCAT
CCTCCCCGCCCACCCTTTAATGGACTAACTACCCTTACCTCAGATTACCGCCCGACCCATCATTATTATATACTT
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CCACACACCCCTTCC

>GHEYSI-A 692bp: SFB2

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TTGGCAGCTCGAGTTTTGTCAGCACACACCTTCATCGGAATGTCACAAAACATGCCCATGTCTATATGCTCTGCC
TCCACCACCCAAATTTTGAACGTCATAACGACACTGATGACCCATATGACATAGAAGAACTTCAGTGGTCACTTT
TTTACAATGAAACGTTCGAGCAGTTCTCCAAGTTAAGCCATCCCTTAGGAAGCACAGAACATTATGGGGTATATG
GTTCAAGCAATGGTTTAATTTGCATTTCGGATGAGATATTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CAATTAGTAAATTTAGGACCCCTCCAATGAGCACCAACATTAACCTTAAATTTGCCTATGTAGCTCTCCAATTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGAATGATGCGTACCAACAAAGATGCCTTCGTGGTTGAGT
TTATAGTCTTCAAACAGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGAGACATCA
TCAAGGTACATTTTTTAACGGAGTAGCATATCACATCATCGAGAAAGGTCTCTATTCAGCATTATGTCATTCGAT
TCAGGCAGTGAAGAAAT

>GHEYSI-D 705bp:SFB*

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TTAACAGCTCGAGTTTTATCACCACACAGCTTAACAGGAATGTCACCAAACACCTCCATGTCTCTCTACTTTGCC
TCCACTACCCCGATCTCAAACGTCCTTTCGAGTTCTACGAAAACTACGAGGACTACCCAGATCTTAAACGAGAAT
TGGAATGGTCACTTTTCTCCAACGAAACATTTGAGCATTGCTCCAAGTTAAACCATCCCTTGGGGATCAAGAAAG
ATTATAGGGCATATGGCTCAAGCAATGGCCTAGTTTGCATTTCGGATGACAAATTGGACACCAAGAGCCCTATAC
ACATATGGAACCCCTCGGTTAGGAAATTTAGAACCCTTCCAATGAGCACCAACGTTAAATTTCGCTACATTGCTC
TCCAATTCGGGTTCCACCCCGGGGTTAACGACTACAAAGTTGTAAGGATGTTGCGCGTCCACAAAGATGATGCTT
TCGCAGTCGAAGTTTATAGTCTCAGCAGAGACTCTTGGAAGATGGTTGAAGAACATCCTCTTTGGTTAAAATGCA
CTTGGCAGACCACAGGGGTACATTTTATAATGGAGTAGCATACCACATTATTGAGAAATTTCCTCTATTCAGCGT
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>NADERI-B 695bp: SFB13

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TCCACCACCCAAATTTTGAACGAAACGACGACCCTGATGACCCATATGTTGAACAAGAATTTCAATGGTCTCATT
TTTCCAATGAAACATTTGAGGAGTGCTCCAAGTTAAGACATCCCTCAGGGAGCACAGAACATTATATGATATATG
GCTCAAGCAATGGCTTAGTTTGCATTTCGGAGGAGATATTGAATTTCGATAGTCCAATACACATATGGAACCCCT
CGGTTAAGAAATTTAGGACCCCTCCAATGAGCACCAACATTAACATTAAATTTAGCTATGTTGCTCTCCAATTCG
GGTTCCACCCCGGGGTTAACGACTACAAGGCTGTAAGGATGATGCGTACCAACAAAAATGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAACAGACTCTTGGAAGATGATTGAAGCAATTCCCCCGTGGTTAAAATGTACTTGGCAGCATC
ATAACGGTACATTTTTTAATGGAGTAGCATACCACGTCCTTCTGAAAGGTCCTATATTCAGCATTATGTCATTTC
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>NADERI-C 685bp: SFB14

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TCCACTACCCCGATCTCAAACGTCCTTTCGAGTTCTACGAAAACTACGAGGACTACCCAGATCTTAAACGAGAAT
TGGAATGGTCACTTTTCTCCAACGAAACATTTGAGCATTGCTCCAAGTTAAACCATCCCTTGGGGATCAAGAAAG
ATTATAGGGTATATGGCTCAAGCAATGGCCTAGTTTGCATTTCGGATGACAAATTGGACACCAAGAGCCCTATAC
ACATATGGAACCCCTCGGTTAGGAAATTTAGAACCCTTCCAATGAGCACCAACGTTAAATTTCGCTACATTGCTC
TCCAATTCGGGTTCCACCCCGGGGTTAACGACTACAAGGTTGTAAGGATGTTGCGCGTCCACAAAGATGATGCTT
TCGCAGTCGAAGTTTATAGTCTCAGCACAGACTCTTGGAAGATGGTTGAAGAACATCCTCTTTGGTTAAAATGCA
CTTGGCAGACCACAGGGGTACATTTTATAATGGAGTAACATACTACATTATTGAGAAATTTCCTCTATTCAGCGT
TTATGTCTTT

>KYOTO-A 694bp: SFB8

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TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
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GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTTGGAACATC
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>KYOTO-B 695bp: SFBC

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TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCCTAAAAAGGAATTTCAATGGTCTCTTT
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GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
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>LILLYCOT-A 693bp: SFB8

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CCACCACCCAAATTTTGAATGTTCGGTCGACCCTGATGATCCAGGTTTTGAACAAGAACTTCAATGGTCTCTTTT
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GTTCCACCCCGGAGTTAATGACTACAAGGCCGTACGAATGATGCTTATTGACAAAGATGCCTTCGCGGTTGAGGT
TTATAGTCTTACCACTGATTCTTGGAAGCTGATTGAAGTAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATCA
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>LILLYCOT-E 748bp* *

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AACGACCATAATGCGGGCTGTTGCCCGGCATCCAACGCCATTCATGGCCATATCAATGATCTCTGGTGCGTACCG
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ACTACAAGGCTGTAAGGATGATGCGTACCAACAAAGATGCCTTGGCGGTTGAGGTTTATAGTCTTAGAACAGACT
CGTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATCATATGGGTACATTTTTTAATG
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>MAYA-A 693bp:SFB8

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TTTCCAATGAAACATTTAAGCTGTGCTCCAAGTTAAGCCATCCCTTAGGGAGTACAGATCGTTATGGGATATATG
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CGGTAAAGAAATTTACGAGCCCTCCAATGAGCACCAACATTAACGTCAAATTTACCTGTGTTGCTCTCCAATTTG
GGTTCCACCCCAGTCTTAATGACTACAAGGTTGTAAGGATGATGCGTACCAACAAAGGTGCCTTGGCGGTTGAGG
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>MAYA-B 694bp: SFB9

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CGGTAAAGAAATTTACGAGCCCTCCAATGAGCACCAACATTAACGTCAAATTTACCTATGTTGCTCTCCAATTTG
GGTTCCACCCCAGTCTTAATGACTACAAGGTTGTAAGGATGATGCGTACCAACAAAGGTGCCTTGGCGGTTGAGG
TTTATACTCTCAGAACGGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
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ATTCAGGCAGTGAAGAAAT

>NINFA-A 696bp: SFBC

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TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTTGCC
TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCATCTCCTCCTTGGTTAAAATGCACTTTGGAACATC
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CGATTCAGGCAGTGAAGAAAT

>NINFA-D 695bp: SFB13

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TTGGGAGCTCCTGTTTTGTCAGCACACACCTTCATAGGAATGTCACAAAACATACCCATGTCTATCTACTATGCC
TCCACCACCCAAATTTTGAACGAAACGACGACCCTGATGACCCATATGTTGAACAAGAATTTCAATGGTCTCTTT
TTTCCAATGAAACATTTGAGGAGTGCTCCAAGTTAAGACATCCCTCAGGGAGCACAGAACATTATATGATATATG
GCTCAAGCAATGGCTTAGTTTGCATTTCGGAGGAGATATTGAATTTCGATAGTCCAATACACATATGGAACCCCT
CGGTTAAGAAATTTAGGACCCCTCCAATGAGCACCAACATTAACATTAAATTTAGCTATGTTGCTCTCCAATTCG
GGTTCCACCCCGGGGTTAACGACTACAAGGCTGTAAGGATGATGCGTACCAACAAAAATGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAACAGACTCTTGGAAGATGATTGAAGCAATTCCCCCGTGGTTAAAATGTACTTGGCAGCATC

* No high sequence similarity (homology) were found in DataBank.

ATAAAGGGTACATTTTTTAATGGAGTAGCATACCACGTCCTTCTGAAAGGTCCTATATTCAGCATTATGTCATTC
GATTCAGGCAGTGAAGAAAT

>PETRA-A 693bp: SFBC?

TCGACATCCTAGTAAGACTACCTGCAAAATCCCTTGTTCGGTTTCTTTGTACATGCAAGTCTTGGAGTGATTTGA
TTGGCAGCTCGAGTTTTGTTAGCATACACCTTAACAGGAATGTCACAGAACATGCCCATGTCTACCTACTCTGCC
TCCTCCACCCAAATTTTGAACGTCTGGCCGACCCTGATGACCCATATGTTAAACAAGAGTTTAGCTGGTCTCTTT
TTTCGAATGAAACATTTGAGGAAAGCTCCAAGATAACCCATCCCTTAGGGAGCACAGAACATTGTGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCAGATGAGATTTTGAATTTCGATAGTCCTATACACATATGGAACCCGT
CGGTTAAGAAATTTAAGACCCCTCCAATGAGCACCAACATTAACATTAAATTTAGCCTCGTTTCTCTCCAATTCG
GGTTCCATCCTAGGGTTAATGACTACAAGGCTGTAAGGATGATGCGCACCAACAAAAATGTCTTGGCGGTTGAGG
TTTATAGTCTCAGCACAGACTCTTGGAAAATGGTTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
ATAACGGTACATTTTTTAATGGAGTAGCATACCACATCATTCAGAAGGTCCTCTATTCAGCATTATGTCCTTCGA
TTCAGGCAGTGAAGAAAT

>PETRA-B 697bp: SFB1

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TCCACCACCCAAATTTTGAACGTCTGGCCGACCCTGATGATCCATATGTTAAGCAAGGATTTCAGTGGTCTCTTT
TTTCTAATGAAACATTTGAGGAGTGCTCCAAGTTAAGCCATCCCTTAGGGAGCAGAGAACCTTATGTGATTTATG
GCTCAAGCAATGGTTTAGTTTGCATTTCCGATGAGATACTGAATTTCGATAGTCCTATACACATATGGAATCCAT
CGGTTAGTAAACTTAGAACCACGCCAATCAGCACCAATATCAGCATTAAATTTAGCCATGTTGCTCTCCAATTCG
GGTTCCACCCCAGGGTTAATGACTTCAAGGCTATAAGGATGTTGCGTACCAACAAAAATGTCTTGGCGGTTGAGG
TTCATAGTCTCAGCACAGACTCTTGGAAAATGGTTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
ATAACGGGTACATTTTTTTAATGGAGTAGCATACCACATCATTCAGAAAGGTCCTCTATTCAGCATTATGTCCTT
TCGATTCAGGCAGTGAAGAAAT

>PINKCOT-A 699bp: SFB8

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CGGTAAAGAAATTTACGAGCCCTCCAATGAGCACCAACATTAACGTCAAATTTACCTATGTTGCTCTCCAATTTG
GGTTCCACCCCAGTCTTAATGACTACAAGGTTGTAAGGATGATGCGTACCAACAAAGGTGCCTTGGCGGTTGACG
TTTATACTCTCAGACCGGACTCCTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
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CTCCGATTCAGGCAGTGAAGAAAT

>PINKCOT-G 694bp: SFB9

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CGGTAAAGAAATTTACGAGCCCTCCAATGAGCACCAACATTAACGTCAAATTTACCTATGTTGCTCTCCAATTTG
GGTTCCACCCCAGTCTTAATGACTACAAGGTTGTAAGGATGATGCGTACCAACAAAGGTGCCTTGGCGGTTGAGG
TTTATACTCTCAGAACGGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
ATAAGGGTATGTTTTTTAAAGGAGTAGCATACAGCATCATTGAGAAAGGTCCTATGTTCAGCATTATGTCATTCG
ATTCAGGCAGTGAAGAAAT

>PORTICI-N 740bp: SFB2

SFB-5F

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CATTTGTCCGTTCAATCACAAAATGCACCCAAATGAATTGAGACCCAGAAACGAGCAGATTCAGAAATTGACAAT
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GTGTAAAAACTAAATCAAGAAATGAATTAAAAAAGGTGGGTGGTGTGTAATAAATAAGAAAAGGGGAAGTCTGCT
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CTTTCGTTTGGGAATTGAGATGCAGAGGTAGAGATTACACACGCAGAGCTTGCCAAATATCAATTTGAAAACGTA
ATTCTGATTCATTCATGACTCATTAGGCTTCTGCGCGCGTCTGCACACATTTCATGGGTTTCACG

>PORTICI-P 595bp: SFB*[*]

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CAGGTCGACCATATGGGAGAGCTCCCAACGCGTTGGATGCATAGCTTGAGTATTCTATAGTGTCACCTAAATAGC
TTGGCGTAATCATGGTCATAGCTGTTTCCTGTGTGAAATTGTTATCCGCTCACAATTCCACACAACATACGAGCC
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GCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTAATGAATCGGCCAACGCGCGGGGAGAGGCGGTTTGCGT
ATTGGGCGCTCTTCCGCTTCCTCGCTCACTGACTCGCTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCT
CACTCAAAGGCGGTAATACGGTTATCCACAGAATCAGGGGATAACGCAGGAAAGAACATGTGAGCAAAAGGCCAG
CAAAAGGCCAGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCCCCCTGACGAGC

>REALE-D 693bp: SFBC

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TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTTGCC
TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTTGGAACATC
ATAGGGGTACGTTTTTCAGTGGAGTAGCATACCACATCATTCAGAAGGTCCTATGCTCAGCATTATGTCATTCGA
TTCAGGCAGTGAAGAAAT

>REALE-E 697bp: SFBC

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TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTTGCC
TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATTATGGGATATATG
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TCATATGGGTACGTTTTTCAACGGATTACCATACCCACATCATTCAGAAAGGTCCTATGCTCAGCACTATGTCAT
TCGATTCAGGCAGTGAAGAAAT

>ROBADA-B 694bp: SFB8

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TTGGCAGCTCGAGTTTTGTTAGCACACATCTTAATAAGAATGTCACAAAACATGCCCATGTCTATCTACTTTGCC
TCCACCACCCAAATTTTGAATGTGTGATCGACCCCGATGACCCATATTTAGAAGAAGAACTTCAATGGTCTCTGT
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TGGTTGGAAGATATAGGACCCCTCCAATGAGCACCAACATTAACATTAAATTTAATTATGTTGCTCTCCAATTCG
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TTTATAGTCTTAGAACAGACTCGTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTGGCAGCATC
ATATGGGTACATTTTTTAATGGAGTAGCATACCATATTATTGAGAAAGGTCCCATATTCAGCATTATGTCCTTCG
ATTCAGGCAGTGAAGAAAT

>ROBADA-D 598bp: SFB22

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TCCACCACCCAAATTTTGAATGTGTGATCGACCCCGATGACCCATATTTAGAAGAAGAACTTCAATGGTCTCTGT
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GTTCAAGCAATGGTTTGCTTTGCATTTCTGATGAGATATTGAATCTCGATAGTCCAATACACATATGGAACCCAT
TGGTTGGAAGATATAGGACCCCTCCAATGAGCACCAACATTAACATTAAAATTTAATTATGTTGCTCTCCAATTC
GGGTTTCACCCTGGGGGTTAATGACTACAAGGCTGTAAAGGATGATGCGTACCAACAAGATGCCCTTGGCGGTTG
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>S.CASTRESE-A 693bp: SFBC

TCGACATCCTAGTAAGACTACCTGCAAAATCCCTTATCCGCTTTCTGAGTACATGCAAGTCGTGGAGTGATTTGA
TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTTGCC
TCCACCTCCCAAATTCTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAACTTCGATAGTCCTATACACATATGGAACCCAT

[*] No high sequence similarity (homology) were found in DataBank.

CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCTTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTTGGAACATC
ATAGGGTACGTTTTTCAGTGGAGTAGCATACCACATCATTCAGAAAGGTCCTATGCTCAGCATTATGTCATTCGA
TTCAGGCAGTGAAGAAAT

>S.CASTRESE-B 694bp: SFBC

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TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTTGCC
TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATCCTGAATTTCGATAGTCCTATACACATATGGAACCCAT
CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCATCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTTGGAACATC
ATAGGGGTACGTTTTTCAGTGGAGTAGCATACCACATCATTCAGAAAGGTCCTATGCTCAGCATTATGTCATTCG
ATTCAGGCAGTGAAGAAAT

>YAMAGATA-C 694bp: SFB8

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TTGGCAGTTCGAGCTTTGTTAGCACACACCTTCATAGGAATGTCAGAAAACATGCCCATGTCTATCTACTTTGCC
TCCACCACCCAAATTTTCGACGTCAGAACGACAATGATGACCCATATGATATAGAAGAACTTCAATGGTCGCTTT
TCTCTAATGAAACATTTGAGCAGTTCTGCGAATTAAGTCATCCCTTAGGGAGCACGGAACATTATGGGATATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATACTGAATTTTGATAGTCCTATACATATATGGAACCCAT
CGGTTAGGAAATTTAGGACCCCTCCAATGAGCACCAACATTAACATGAAATTTTCCCATGTTGCTCTCCAATTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGTTGTAAGGATGATGCGTACCAACAAAGGTGCCTTGGCAGTTGAGG
TATATAGTCTTAGAACAGACTGTTGGAAAATGATTGAAACAATTCCTCCTTGGTTAAAATGCACTTGGCAACATC
ATAAGGGCAAATTTTTTAATGGAGTAGCATACCACGTCATTAAGAAAGGTCCTATATTCAGCATTATGTCCTTCG
ATTCAGGCAGTGAAGAAAT

>YAMAGATA-D 694bp: SFB8

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TTGGCAGTTCGAGTTTTGTTAGCACACACCTTCATAGGAATGTCAGAAAACATGCCCATGTCTATCTACTTTGCC
TCCACCACCCAAATTTTCGACGTCAGAACGACAATGATGACCCATATGATATAGAAGAACTTCAATGGTCGCTTT
TCTCTAATGAAACATTTGAGCAGTTCTGCGAATTAAGTCATCCCTTAGGGAGCACGGAACATTATGGGGTATATG
GTTCAAGCAATGGTTTAGTTTGCATTTCGGATGAGATACTGAATTTTGATAGTCCTATACATATATGGAACCCAT
CGGTTAGGAAATTTAGGACCCCTCCAATGAGCACCAACATTAACATGAAATTTTCCCATGTTGCTCTCCAATTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGTTGTAAGGATGATGCGTACCAACAAAGGTGCCTTGGCAGTTGAGG
TATATAGTCTTAGAACAGACTGTTGGAAAATGATTGAAACAATTCCTCCTTGGTTAAAATGCACTTGGCAACATC
ATAAGGGCAAATTTTTTAATGGAGTAGCATACCACGTCATTAAGAAAGGTCCTATATTCAGCATTATGTCCTTCG
ATTCAGGCAGTGAAGAAAT

>LITO-E 694bp: SFBC

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TTGGAAGCTCAATTTTTGTTAGCACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTTGCC
TCCACCACCCAAATTTTGAGCGTTTGGTCGACCCTAATGACCCATATCTTAAAAAGGAATTTCAATGGTCTCTTT
TTCCCAAAGAAACATTTGAGGAGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATTATGGGATATATG
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CGGTTAGGAAATTTAGGGCCCTTCCAATGAGCACCAACATTAACATTAAATTTAGCTGTGTTGCTCTCCAGTTCG
GGTTCCACCCTGGGGTTAATGACTACAAGGCTGTAAGGATGATGCGTACCAATAAAAGTGCCTTGGCGGTTGAGG
TTTATAGTCTCAAAAGAGACTCTTGGAAGATGATTGAAGCAATTCCTCCTTGGTTAAAATGCACTTTGGAACATC
ATAGGGGTACGTTTTTCAGTGGAGTAGCATACCACATCATTCAGAAAGGTCCTATGCTCAGCATTATGTCATTCG
ATTCAGGCAGTGAAGAAAT

Appendix B: Alignments

•Alignment obtained for *S-RNases* in this work.

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NADERI1           CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC------G 54
NINFA1            CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC------T 54
 MAYA1             ------------------------------------------------------------
PORTICI1          -----CTCGCTTTCCTTGTTCTTGCTTTTCTTCTTGTGTTTCATAATGAGCACTGGTGAT 55
PORTICI2          ----CTCGCTTTCCTTGTTCTTGCTTTTGCTTCTTGTGTTTCATAATGAGCACTGGTGAT 56
GHEYSI2           ------CTCGCTTTCCTTGTTCTTGCTTTTTTCTTGTGTTTCATAATGAGCACTGGTGAT 54
BO92639095-2      CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
13F-PETRA         CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
CORNIA2           CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
CORNIA1           CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
BO93622312        CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
AITERA1           CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
LILYCOT2          CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
MAYA2             CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTTTGTTACGTTATGAGCA------GT 54
GHEYSI1           CTCGCTTTCCTTGTTCTTGCTTTTGCTATCTTCTTGTGTTTCATTATGAGCACTGGTGAT 60
AROBADA1          CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCA------CT 54
T.DI              CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCA------CT 54
KYOTO2            CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC---TAGT 57
LITO1             CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC---TAGT 57
NINFA2            CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC---TAGT 57
Bora1             CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC---TAGT 57
BO92639095-1      CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC---TAGT 57
ALTERA2           CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC---TAGT 57
KYOTO1            CTCGCTTTCCTTGTTCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC---TAGT 57
 NADERI2           ------------------------------------------------------------
LILYCOT1          CTCGCTTTCCTTGTTCTTGCTTTTGTCTTGTTCTTGTGTTTCAGTATGAGCAC------T 54
 1PETRA1           ------------------------------------------------------------
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 NADERI1           GGTGGGTGGCATTATAATTGCTTTGCTCTTTGTATTCTGC----------CATGTAGTCAG 105
 NINFA1            GGTGGGTTGCATTATAATTTCTTTGCTCTTTGTATTCTGC----------CATATAGTCAG 105
 MAYA1             ----------------------------------------GT--ATGTGCATATAATTTG 18
 PORTICI1          GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATGCCATGT--ATGTGCATATAATTTG 112
 PORTICI2          GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATGCCATGT--ATGTGCATATAATTTG 113
 GHEYSI2           GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATGCCATGT--ATGTGCATATAATTTG 111
 BO92639095-2      GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATATCCTGTATATATGCATATAATCAG 113
 13F-PETRA         GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATATCCTGTATATATGCATATAATCAG 113
 CORNIA2           GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATATCCTGTATATATGCATATAATCAG 113
 CORNIA1           GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATATCCTGTATATATGCATATAATCAG 113
 BO93622312        GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATATCCTGTATATATGCATATAATCAG 113
 AITERA1           GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATATCCTGTATATATGCATATAATCAG 113
 LILYCOT2          GGTGGGTTGCATTACAATC-TTT-GCTCTTTATATCCTTT------ATGCATATAATCAG 106
 MAYA2             GGTGGGTTGCATTACAATC-TTT-GCTCTTTATATCCTTT------ATGCATATAATCAG 106
 GHEYSI1           GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCCTAT------ATGCATATAATCAG 113
 AROBADA1          GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATTATATCC-TGGATGCATATAATTAG 112
 T.DI              GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATTATATCC-TGGATGCATATAATTAG 112
 KYOTO2            GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCATAT------ATGCATATAATTAG 110
 LITO1             GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCATAT------ATGCATATAATTAG 110
 NINFA2            GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCATAT------ATGCATATAATTAG 110
 Bora1             GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCATAT------ATGCATATAATTAG 110
 BO92639095-1      GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCATAT------ATGCATATAATTAG 110
 ALTERA2           GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCATAT------ATGCATATAATTAG 110
 KYOTO1            GGTGGGTTGCATTACAATC-TTTTGCTCTTTATATCATAT------ATGCATATAATTAG 110
 NADERI2           ------------------------------------------------------------
 LILYCOT1          GGTGGGTTGCATTATAAAT-ATTTGTGCTCGATG------------GTATATGTACTTTG 101
 1PETRA1           ------------------------------------------------------------
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NADERI1          TATTGCATTTTA-TACTTAAATTTTATGTTTAGAGAAATATATATATGCATT-------- 156
NINFA1           TATTGCATTTTA-TACTTAAATTTTATGTTTAGAGAAATATATATATGCATT-------- 156
MAYA1            CATTGAATTTTTCT--------------------ACTTCTATTTTATGTGTG-------- 50
PORTICI1         CATTGAATTTTTCT--------------------ACTTCTATTTTATGTGTG-------- 144
PORTICI2         CATTGAATTTTTCT--------------------ACTTCTATTTTATGTGTG-------- 145
GHEYSI2          CATTGAATTTTTCT--------------------ACTTCTATTTTATGTGTG-------- 143
BO92639095-2     CATTACATTTTTCTAATTGTATTTTTTGTTGAGAGAAACTATATTGTGAGTGTTCAATGA 173
PETRA            CATTACATTTTTCTAATTGTATTTTTTGTTGAGAGAAACTATATTGTGAGTGTTCAATGA 173
CORNIA2          CATTACATTTTTCTAATTGTATTTTTTGTTGAGAGAAACTATATTGTGAGTGTTCAATGA 173
CORNIA1          CATTACATTTTTCTAATTGTATTTTT-GTTGAGAGAAACTATATTGTGAGTGTTCAATGA 172
BO93622312       CATTACATTTTTCTAATTGTATTTTTTGTTGAGAGAAACTATATTGTGAGTGTTCAATGA 173
AITERA1          CATTACATTTTTCTAATTGTATTTTTTGTTGAGAGAACCTATATTGTGAGTGTTCAATGA 173
LILYCOT2         CATTCTGTTGT-------GTGTGCTCGAT---GATATATCACATTACATGTG-------G 149
MAYA2            CATTCTGTTGT-------GTGTGCTCGAT---GATATATCACATTACATGTG-------G 149
GHEYSI1          CATTGCATTTTTCTAATTGTATTTT--GTTGACAGAAACTATTGTGTGGATGATATATTA 171
AROBADA1         CATTGCATTTTTCTACTTCTATTTTATGTCTAGAGATA-TATTGTGTGTGTGATGATATA 171
T.DI             CATTGCATTTTTCTACTTCTATTTTATGTCTAGAGATA-TATTGTGTGTGTGATGATATA 171
KYOTO2           AATTACG----------------------AAGGAGAAGTAGGCAGGAAACG-------- 139
LITO1            AATTACG----------------------AAGGAGAAGTAGGCAGGAAATG-------- 139
NINFA2           AATTACG----------------------AAGGAGAAGTAGGCAGGAAATG-------- 139
Bora1            AATTACG----------------------AAGGAGAAGTAGGCAGGAAATG-------- 139
BO92639095-1     AATTACG----------------------AAGGAGAAGTAGGCAGGAAATG-------- 139
ALTERA2          AATTACG----------------------AAGGAGAAGTAGGCAGGAAATG-------- 139
KYOTO1           AATTACG----------------------AAGGAGAAGTAGGCAGGAAATG-------- 139
NADERI2          ------------------------------------------------------------
LILYCOT1         TTATTTAT---------------------------------TACATTACCATTG-------- 122
1PETRA1          ------------------------------------------------------------


NADERI1          --------------------------TATCAGGTAAGGGAGGACTTGATTTAGCGCACAA 190
NINFA1           --------------------------TATCAGGTAAGGGAGGACTTGATCTAGCGCACAA 190
MAYA1            ------------------------------------------GAT---AACTATTGTATG- 65
PORTICI1         ------------------------------------------GAT---AACTATTGTATG- 159
PORTICI2         ------------------------------------------GAT---AACTATTGTATG- 160
GHEYSI2          ------------------------------------------GAT---AACTATTGTATG- 158
BO92639095-2     TATAT-CACATGACATGCG----GTCCACCCAC-TATTTTTCATTTAATCTAGCGCACAA 227
13F-PETRA        TATAT-CACATGACATGCC----GTCCACCCAC-TATTTTTCATTTAATCTAGCGCACAA 227
CORNIA2          TATAT-CACATGACATGCG----GTCCACCCAC-TATTTTTCATTTAATCTAGCGCACAA 227
CORNIA1          TATAT-CACATGACATGCG----GTCCACCCAC-TATTTTTCATTTAATCTAGCGCACAA 226
BO93622312       TATAT-CACATGACATGCG----GTCCACC--C-TATTTTTCATTTAATCTAGCGCACAA 225
AITERA1          TATAT-CACATGACATGCG----GTCCACCCAC-TATTTTTCATTTAATCTAGCGCACAA 227
LILYCOT2         TGTAT-TGCAT--------------TCACCCGCATATTTTTCATTTAATCTAACGCGCAA 194
MAYA2            TGTAT-TGCAT--------------TCACCCGCATATTTTTCATTTAATCTAACGCGCAA 194
GHEYSI1          CATGA-AACATGCGGTGTATTGAATTCACCCACATATTTTTCATTTAATCTAAAGCACAA 230
AROBADA1         TATATATATAT-ATATATAT---ATATATCAGGTAATGGAGGACTTGATCTAGCGCACAC 227
T.DI             TATATATATAT-ATATATAT---ATATATCAGGTAATGGAGGACTTGATCTAGCGCACAC 227
KYOTO2           ----------------------------------------TCATT--AATTAGTACATAA 157
LITO1            ----------------------------------------TCATT--AATTAGTACATAA 157
NINFA2           ----------------------------------------TCATT--AATTAGTACATAA 157
Bora1            ----------------------------------------TCATT--AATTAGTACATAA 157
BO92639095-1     ----------------------------------------TCATT--AATTAGTACATAA 157
ALTERA2          ----------------------------------------TCATT--AATTAGTACATAA 157
KYOTO1           ----------------------------------------TCATT--AATTAGTACATAA 157
NADERI2          ------------------------------------------------------------
LILYCOT1         ----------------------------------------CGTTTCTACTTGTATTTTCA 142
1PETRA1          ------------------------------------------------------------
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NADERI1          CGTTCTTTGGATGAATAACTATTTGGGAATTACATTTCTG-CATGGTTTCTGTGGTCTAC 249
NINFA1           CGTTCTTTGGATGAATAACTATTTGGGAATTACATTTCTG-CATGGTTTCTTTGGTCTAC 249
MAYA1            ----TTTTCGATGA---------TGGGGAGGACTTATATAGCGCACAACTTTCTTTACTC 112
PORTICI1         ----TTTTCGATGA---------TAGGGAGGACTTATATAGCGCACAACTTTCTTTACTC 206
PORTICI2         ----TTTTCGATGA---------TAGGGAGGACTTATATAGCGCACAACTTTCTTTACTC 207
GHEYSI2          ----TTTTCGATGA---------TAGGGAGGACTTATATAGCGCACAACTTTCTTTACTC 205
BO92639095-2     CTTTCTTTGGATGAGTAAGTATTTGGGGATTGTTTTTCCAGCATGTTCTCTTTTTATTTT 287
13F-PETRA        CTTTCTTTGGATGACTAAGTATTTGGGGATTGTTTTTCCAGCATGTTCTCTTTTTATTTT 287
CORNIA2          CTTTCTTTGGATGAGTAAGTATTTGGGGATTGTTTTTCCAGCATGTTCTCTTTTTATTTT 287
CORNIA1          CTTTCTTTGGATGAGTAAGTATTTGGGGATTGTTTTTCCAGCATGTTCTCTTTTTATTTT 286
BO93622312       CTTTCTTTGGATGAGTAAGTATTTGGGGATTGTTTTTCCAGCATGTTCTCCTTTTATTTT 285
AITERA1          CTTTCTTTGGATGAGTAAGTATTTGGGGATTGTTTTTCCAGCATGTTCTCTTTTTATTTT 287
LILYCOT2         CTTTCTTTGGATGAGTA------TAGGGATTGCTTTTCTT-CATGTCCTC-TTCTATTTT 246
MAYA2            CTTTCTTTGGATGAGTA------TAGGGATTGCTTTTCTT-CATGTCCTC-TTCTATTTT 246
GHEYSI1          CTTTCTTTGGATAAGTAAGTATTTGGGGATTATTTCTCTA-------------------C 271
AROBADA1         CTTTCTTTGGATGAGAAACTGTTTGGGATTTTTTTTTATTTTTTATTTCTGCATGGATTC 287
T.DI             CTTTCTTTGGATGAGAAACTGTTTGGGATTTTTTTTTATTTTTTATTTCTGCATGGATTC 287
KYOTO2           CTTTCTTTGGATGAGTTACTATTTGGGAATTATTTTTCTG-CATGGTATCTTTCGATTAC 216
LITO1            CTTTCTTTGGATGAGTTACTATTTGGGAATTATTTTTCTG-CATGGTATCTTTCGATTAC 216
NINFA2           CTTTCTTTGGATGAGTTACTATTTGGGAATTATTTTTCTG-CATGGTATCTTTCGATTAC 216
Bora1            CTTTCTTTGGATGAGTTACTATTTGGGAATTATTTTTCTG-CATGGTATCTTTCGATTAC 216
BO92639095-1     CTTTCTTTGGATGAGTTACTATTTGGGAATTATTTTTCTG-CATGGTATCTTTCGATTAC 216
ALTERA2          CTTTCTTTGGATGAGTTACTATTTGGGAATTATTTTTCTG-CATGGTATCTTTCGATTAC 216
KYOTO1           CTTTCTTTGGATGAGCTACTATTTGGGAATTATTTTTCTG-CATGGTATCTTTCGATTAC 216
NADERI2          CTCGCTTTCCTTGT-------TCTTGCTTTTGCTTTCTTCTTGTGTTTCATTATGAGCAC 53
LILYCOT1         CTCGGATCAAGTAACTATTTGTTTGGGAATTATTTTCTAC-------------------AT 184
1PETRA1          CTCGCTTTCCTTGT-------TCTTGCTTTTCTTTCCTTGTTCTTGCCTATTCCTCCCGT 53
*    *           * *        *
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NADERI1          TTTGATTGTTGTTGC-AATAAGTGCAGTGTTCATCATTTCAAGCTAAAACTATGTACTTT 308
NINFA1           TTTGATTGTTGTTGC-AATAAGTGCAGTGTTCATCATTTCAAGCTAAAACTAGGTACTTT 308
MAYA1            TGATAGTGTTGCA----ATAAGTACAGTATTCATCATTAGAAACCTTATTGAAGAT---- 164
PORTICI1         TGATAGTGTTGCA----ATAAGTACAGTATTCATCATTGGAAACCTTATTGAAGAT---- 258
PORTICI2         TGATAGTGTTGCA----ATAAGTACAGTATTCATCATTGGAAACCTTATTGAAGAT---- 259
GHEYSI2          TGATAGTGTTGCA----ATAAGTACAGTATTCATCAT-GGAAACCTTATTGAAGAT---- 256
BO92639095-2     CATCCTCTTTACT-----TTATTATGATAATTG---TTGCAACTTGCAATAA-GT----- 333
13F-PETRA        C-TCCTCTTTACT-----TTATTATGATAATTG---TTGCAACTTGCA-TAC-GT----- 331
CORNIA2          CATCCTCTTTACT-----TTATTATGATAATTG---TTGCAACTTGCAATAA-GT----- 333
CORNIA1          CATCCTCTTTACT-----TTATTATGATAATTG---TTGCAACTTGCAATAA-GT----- 332
BO93622312       CATCCTCTTTACT-----TTATTATGATAATTG---TTGCAACTTGCAATAA-GT----- 331
AITERA1          CATCCTCTTTACT-----TTATTATGATAATTG---TTGCAACTTGCAATAA-GT----- 333
LILYCOT2         CAGCCTCTTTTGT-----TTTTTCTGATAAT-----TTG----TTGCAATAA-GT----- 286
MAYA2            CAGCCTCTTTTGT-----TTTTTCTGATAAT-----TTG----TTGCAATAA-GT----- 286
GHEYSI1          AGTCCTCTTTCGT-----TTGTTCTGGTAAT------TG----TTCCAATAA-GT----- 310
AROBADA1         TTTCGTTTACCGTGATAGTTGTTGCAATAAGTGCATTTGAAGCTAAAATTAT-GTTGTT- 345
T.DI             TTTCGTTTACCGTGATAGTTGTTGCAATAAGTGCATTTGAAGCTAAAATTAT-GTTGTT- 345
KYOTO2           TCTGATAGTTGTTGA-AATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTC 275
LITO1            TCTGATAGTTGTTGA-AATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTC 275
NINFA2           TCTGATAGTTGTTGA-AATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTC 275
Bora1            TCTGATAGTTGTTGA-AATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTC 275
BO92639095-1     TCTGATAGTTGTTGA-AATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTC 275
ALTERA2          TCTGATAGTTGTTGA-AATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTC 275
KYOTO1           TCTGATAGTTGTTGA-AATAAGTGCAGTATTCATCATTGGAAGCTAAAATGGTGTTCTTC 275
NADERI2          TGGTGGGTTGCATTA---CAATCTTTTTGCTCGTCATTTCAAGCTAAAACTATGTACTTT 110
LILYCOT1         TGTTACTTTTG--------TGTTTCAGTA------------AGCTAAAATTATGT----- 219
1PETRA1          GCCAATGATTTCC-----TAACTGGGTTCATCTCTTTCCCTTGCTTTTCTTATATCTGGA 108
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NADERI1           ATACATCAAATCCTTATTTAAGATGCCATTAACCTTCTCACAATAATTTGCGCGGGATCT 368
NINFA1            ATACATCAAATCCTTATTTAAGATACCATTAACCTTCTCAAAATAATTTTCGCAGGATCT 368
MAYA1             --ACCATTAACCT--------------TTTA------TCACAATAATTTTGGCAGGAACT 202
PORTICI1          --ACCATTAACCT--------------TTTA------TCACAATAATTTTGGCAGGAACT 296
PORTICI2          --ACCATTAACCT--------------TTTA------TCACAATAATTTTGGCAGGAACT 297
GHEYSI2           --ACCATTAACCT--------------TTTA------TCACAATAATTT-GGCAGGAACT 293
BO92639095-2      --GCA---G-TCT--------------GTTCA-----TCACAATAATTTTGGCAGGATCT 368
13F-PETRA         --GCA---G-TCT--------------GTTCA-----TCACAATAATTTTGGC-GGATCT 365
CORNIA2           --GCA---G-TCT--------------GTTCA-----TCACAATAATTTTGGCAGGATCT 368
CORNIA1           --GCA---G-TCT--------------GTTCA-----TCACAATAATTTTGGCAGGATCT 367
BO93622312        --GCA---G-TCT--------------GTTCA-----TCACAATAATTTTGGCAGGATCT 366
AITERA1           --GCA---G-TCT--------------GTTCA-----TCACAATAATTTTGGCAGGATCT 368
LILYCOT2          --GCA---GGCCT--------------ATTCA-----TCACAATAATTTTGGCAGGATCT 322
MAYA2             --GCA---GGCCT--------------ATTCA-----TCACAATAATTTTGGCAGGATCT 322
GHEYSI1           --GCA---G-TCT--------------ATTCA-----TCACGATAATTTTGGCAGGATCT 345
AROBADA1          -TGAG---ATACC--------------ATCAACCTTCTCACAATAATTTTGGCAGGATCT 387
T.DI              -TGAG---ATACC--------------ATCAACCTTCTCACAATAATTTTGGCAGGATCT 387
KYOTO2            CTGCATAAAATCC--------------ATTAACCTTCTCACAATAATTTTCGCAGGATCT 321
LITO1             CTGCATAAAATCC--------------ATTAACCTTCTCACAATAATTTTCGCAGGATCT 321
NINFA2            CTGCATAAAATCC--------------ATTAACCTTCTCACAATAATTTTCGCAGGATCT 321
Bora1             CTGCATAAAATCC--------------ATTAACCTTCTCACAATAATTTTCGCAGGATCT 321
BO92639095-1      CTGCATAAAATCC--------------ATTAACCTTCTCACAATAATTTTCGCAGGATCT 321
ALTERA2           CTGCATAAAATCC--------------ATTAACCTTCTCACAATAATTTTCGCAGGATCT 321
KYOTO1            CTGCATAAAATCC--------------ATTAACCTTCTCACAATAATTTTCGCAGGATCT 321
NADERI2           ATACATCAAATCCTTATTTAAGATGCCATTAACCTCCTCACAATAATTTGCGCAGGATCT 170
LILYCOT1          ----------TCT-----------------------------------TTATGCAGGATCT 235
1PETRA1           CAATCGTTTATTTT-------------GTGAAGGAAGTCACA-----TGGAGATGGAGCT 150
*   *  *** **
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NADERI1           TACGTGTATTTTCAATTTGTGCAACAA-GGCC 399
NINFA1            TATGTGTATTTTCAATTTGTGCAACAATGGCC 400
MAYA1             TATGACTATTTCCAATTTGTGCAACAATGGCC 234
PORTICI1          TATGACTATTTCCAATTTGTGCAACAATGGCC 328
PORTICI2          TATGACTATTTCCAATTTGTGCAACAATGGCC 329
GHEYSI2           TATGACTATTTCCAATTTGTGCAACAA-GGCC 324
BO92639095-2      TATGATTATTTTCAATTTGTGCAACAATGGCC 400
13F-PETRA         TATGATTATTTTCAATTTGTGCAACAATGGCC 397
CORNIA2           TATGATTATTTTCAATTTGTGCAACAATGGCC 400
CORNIA1           TATGATTATTTTCAATTTGTGCAACAATGGCC 399
BO93622312        TATGATTATTTTCAATTTGTGCAACAATGGCC 398
AITERA1           TATGATTATTTTCAATTTGTGCAACAATGGCC 400
LILYCOT2          TATGACTATTTTCAATTTGTGCAACAATGGCC 354
MAYA2             TATGACTATTTTCAATTTGTGCAACAATGGCC 354
GHEYSI1           TATGACTATTTTCAATTTGTGCAACAA-GGCC 376
AROBADA1          TATGTCTATTTTCAATTTGTGCAACAATGGCC 419
T.DI              TATGTCTATTTTCAATTTGTGCAACAATGGCC 419
KYOTO2            TATGTCTATTTTCAATTTGTGCAACAATGGCC 353
LITO1             TATGTCTATTTTCAATTTGTGCAACAATGGCC 353
NINFA2            TATGTCTATTTTCAATTTGTGCAACAATGGCC 353
Bora1             TATGTCTATTTTCAATTTGTGCAACAATGGCC 353
BO92639095-1      TATGTCTATTTTCAATTTGTGCAACAATGGCC 353
ALTERA2           TATGTCTATTTTCAATTTGTGCAACAATGGCC 353
KYOTO1            TATGTCTATTTTCAATTTGTGCAACAATGGCC 353
NADERI2           TACGTGTATTTTCAATTTGTGCAACAATGGCC 202
LILYCOT1          TATCAATATTTTCAATTTGTGCAACAATGGCC 267
1PETRA1           CA--ATTTTTATCAATTTGTGCAACAATGGCC 180
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•Alignment obtained for *SFBs* in this work.

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GHEYSI-D          TCGACATCCTAGTAAGACTACCTGCAAAAA 30
NADERI-C          TCGACATCCTAGTAAGACTACCTGCAAAAA 30
Altera-E          TCGACATCCTAGTAAGACTACCTGCAAAAA 30
KYOTO-B           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
REALE-D           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
KYOTO-A           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
S.CASTRESE-B      TCGACATCCTAGTAAGACTACCTGCAAAAT 30
LITO-E            TCGACATCCTAGTAAGACTACCTGCAAAAT 30
BORA-A            TCGACATCCTAGTAAGACTACCTGCAAAAT 30
S.CASTRESE-A      TCGACATCCTAGTAAGACTACCTGCAAAAT 30
Altera-F          TCGACATCCTAGTAAGACTACCTGCAAAAT 30
NINFA-A           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
REALE-E           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
BO92639095-B      TCGACATCCTAGTAAGACTACCTGCAAAAT 30
CORNIA-C          TCGACATCCTAGTAAGACTACCTGCAAAAT 30
PETRA-B           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
BO93622312-F      TCGACATCCTAGTAAGACTACCTGCAAAAT 30
CORNIA-B          TCGACATCCTAGTAAGACTACCTGCAGAAT 30
PETRA-A           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
NADERI-B          TCGACATCCTAGTAAGACTACCTGCAAAAT 30
NINFA-D           TCGACATCCTAGTAAGACTACCTGCAAAAT 30
BORA-B            TCGACATCCTAGTAAGACTACCTGCAAAAT 30
MAYA-B            TCGACATCCTAGTAAGACTACCTGCAAAAT 30
PINKCOT-G         TCGACATCCTAGTAAGACTACCTGCAAAAT 30
PINKCOT-A         TCGACATCCTAGTAAGACTACCTGCAAAAT 30
MAYA-A            TCGACATCCTAGTAAGACTACCTGCAAAAT 30
YAMAGATA-C        TCGACATCCTAGTAAGACTACCTGCAAAAT 30
YAMAGATA-D        TCGACATCCTAGTAAGACTACCTGCAAAAT 30
GHEYSI-A          TCGACATCCTAGTAAGACTACCTGCAAAAT 30
ROBADA-B          TCGACATCCTAGTAAGACTACCTGCAAAAT 30
ROBADA-D          TCGACATCCTAGTAAGACTACCTGCAAAAT 30
LILLYCOT-A        TCGACATCCTAGTAAGACTACCTGCAAAAT 30
LILLYCOT-E        TAGCGTAAAGAGCTGTGCTGGTGGTTTTAGCCCAGA-CATAGCCCCACTGATCGTCATTT 59
PORTICI-N         --------------------TAGGACCCCTCCAATGAGCCCTTTATTTTCTGTTGAATGC 40
PORTICI-P         ------------------------------------------------------------
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GHEYSI-D          CCCTTGTTCGATTTC-TGTGTGCATGCAAGTCATG-GAGTGATTTGATTAACAGCTCGAG 88
NADERI-C          CCCTTGTTCGATTTC-TGTGTGCATGCAAGTCATG-GAGTGATTTGATTAACAGCTCGAG 88
Altera-E          CCCTTGTTCGATTTC-TGTGTGCATGCAAGTCATG-GAGTGATTTGATTAACAGCCCGAG 88
KYOTO-B           CCCTTATTCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
REALE-D           CCCTTATTCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
KYOTO-A           CCCTTATTCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
S.CASTRESE-B      CCCTTATTCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
LITO-E            CCCTTATTCGCTTTC-TGAGTACGTGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
BORA-A            CCCTTATTCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
S.CASTRESE-A      CCCTTATCCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
Altera-F          CCCTTATTCGCTTTC-CGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
NINFA-A           CCCTTATTCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
REALE-E           CCCTTATTCGCTTTC-TGAGTACATGCAAGTCGTG-GAGTGATTTGATTGGAAGCTCAAT 88
BO92639095-B      CCCTTGTTCGGTTTC-TTTGTACATGCAAGTCTTG-GAGTGATTTGATTGGCAGCTCGAG 88
CORNIA-C          CCCTTGTTCGGTTTC-TTTGTACATGCAAGTCTTG-GAGTGATTTGATTGGCAGCTCGAG 88
PETRA-B           CCCTTGTTCGGTTTC-TTTGTACATGCAAGTCTTG-GAGTGATTTGATTGGCAGCTCGAG 88
BO93622312-F      CTCTCGTTCGGTTTC-TGTGTACATGCAAGTCATG-GAGTGGTTTGATTGGCAGCTCGAG 88
CORNIA-B          CTCTCGTTCGGTTTC-TGTGTACATGCAAGTCATG-GAGTGGTTTGATTGGCAGCTCGAG 88
PETRA-A           CCCTTGTTCGGTTTC-TTTGTACATGCAAGTCTTG-GAGTGATTTGATTGGCAGCTCGAG 88
NADERI-B          CCCTTGTTCGGTTTC-TGTGTACGTGCAAGTCATG-GAGTGATTTGATTGGGAGCTCCTG 88
NINFA-D           CCCTTGTTCGGTTTC-TGTGTACGTGCAAGTCATG-GAGTGATTTGATTGGGAGCTCCTG 88
BORA-B            CCCTCGTTCGGTTTC-TGTGTACATGCAAGTCATG-GAGTGATTTGATTAGCAGCCCGAG 88
MAYA-B            CCCTCGTTCGGTTTC-TGTGTACATGCAAGTCATG-GAGTGATTTGATTAGCAGCCCGAG 88
PINKCOT-G         CCCTCGTTCGGTTTC-TGTGTACATGCAAGTCATG-GAGTGATTTGATTAGCAGCCCGAG 88
PINKCOT-A         CCCTCGTTCGGTTTC-TGTGTACATGCAAGTCATG-GAGTGATTTGATTAGCAGCCCGAG 88
MAYA-A            CCCTCGTTCGGTTTC-TGTGTACATGCAAGTCATG-GAGTGATTTGATTAGCAGCCCGAG 88
YAMAGATA-C        CCCTCGTTCGGTTTC-TTTGTACATGCAAGTCATG-GAGTGATTTGATTGGCAGTTCGAG 88
YAMAGATA-D        CCCTCGTTCGGTTTC-TTTGTACGTGCAAGTCATG-GAGTGATTTGATTGGCAGTTCGAG 88
GHEYSI-A          CCCTCGTTCGGTTTC-TTTGTACATGCAAGTCATG-GAGTGATTTGATTGGCAGCTCGAG 88
ROBADA-B          CCCTCATTCGGTTTC-TAAGTACATGCAAGTCATG-GAGTGATTTGATTGGCAGCTCGAG 88
ROBADA-D          CCCTCATTCGGTTTC-TAAGTACATGCAAGTCATG-GAGTGATTTGATTGGCAGCTCGAG 88
LILLYCOT-A        CACTCGTTCGATTTC-TGTGTACATGCAAAT-ATG-GAGTGATTTGATAAGCAGCTCGAG 87
LILLYCOT-E        CGCGCAGACGATGACGTCACTGCCCGGTCCGTATGCGCGAGGTTACAGACACGGCATGAG 119
PORTICI-N         TCCGCAGACAGCTCTCCTTTCCCATCAAGACCA---GACTGATTACGCAAAGCACCAAGG 97
PORTICI-P         ------------------------------------------------------TAGG 4
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GHEYSI-D          TTTTATCACC-ACACAGCTTAACAGGAATGTCACCAAACACCTCCATGTCTCTCTACTTT 147
NADERI-C          TTTTATCACC-ACACAGCTTAACAGGAATGTCACCAAACACCTCCATGTCTCTCTACTTT 147
Altera-E          TTTTATCACC-ACACAGCTTAACAGGAATGTCACCAAACACCTCCATGTCTCTCTACTTT 147
KYOTO-B           TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
REALE-D           TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
KYOTO-A           TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
S.CASTRESE-B      TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
LITO-E            TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
BORA-A            TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
S.CASTRESE-A      TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
Altera-F          TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAACCATACCCATGTTTATCTACTTT 147
NINFA-A           TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
REALE-E           TTTTGTTAGC-ACACACCTTTGTAGGAATGTGACAAAACATACCCATGTTTATCTACTTT 147
BO92639095-B      TTTTGTTAGC-GTACACCTTAACAGGAACGTCAAAAAGCACGCCAATGTTTATCTACTCT 147
CORNIA-C          TTTTGTTAGC-GTACACCTTAACAGGAACGTCAAAAAGCACGCCAATGTTTATCTACTCT 147
PETRA-B           TTTTGTTAGC-GTACACCTTAACAGGAACGTCAAAAAGCACGCCAATGTTTATCCACTCT 147
BO93622312-F      TTTTGTTAGC-ATACACCTTAACAGGAATGTCACAGAACATGCCCATGTCTATCTACTCT 147
CORNIA-B          TTTTGTTAGC-ATACACCTTAACAGGAATGTCACAGAACATGCCCATGTCTATCTACTCT 147
PETRA-A           TTTTGTTAGC-ATACACCTTAACAGGAATGTCACAGAACATGCCCATGTCTACCTACTCT 147
NADERI-B          TTTTGTCAGC-ACACACCTTCATAGGAATGTCACAAAACATACCCATGTCTATCTACTAT 147
NINFA-D           TTTTGTCAGC-ACACACCTTCATAGGAATGTCACAAAACATACCCATGTCTATCTACTAT 147
BORA-B            TTTTGTCAGC-ACACACCTTTATAGGAATGTCACAAAACATGCCCATGTCTATCTACTTT 147
MAYA-B            TTTTGTCAGC-ACACACCTTTATAGGAATGTCACAAAACATGCCCATGTCTATCTACTTT 147
PINKCOT-G         TTTTGTCAGC-ACACACCTTTATAGGAATGTCACAAAACATGCCCATGTCTATCTACTTT 147
PINKCOT-A         TTTTGTCAGC-ACACACCTTTATAGGAATGTCGCAAAACATGCCCATGTCTATCTACTTT 147
MAYA-A            TTTTGTCAGC-ACACACCTTTATAGGAATGTCACAAAACATGCCCATGTCTATCTACTTT 147
YAMAGATA-C        CTTTGTTAGC-ACACACCTTCATAGGAATGTCAGAAAACATGCCCATGTCTATCTACTTT 147
YAMAGATA-D        TTTTGTTAGC-ACACACCTTCATAGGAATGTCAGAAAACATGCCCATGTCTATCTACTTT 147
GHEYSI-A          TTTTGTCAGC-ACACACCTTCATCGGAATGTCACAAAACATGCCCATGTCTATATGCTCT 147
ROBADA-B          TTTTGTTAGC-ACACATCTTAATAAGAATGTCACAAAACATGCCCATGTCTATCTACTTT 147
ROBADA-D          TTTTGTTAGC-ACACATCTTAATAAGAATGTCACAAAACATGCCCATGTCTATCTACTTT 147
LILLYCOT-A        TTTTGTTAGC-ACACACCTAAACAGGAATGTCACAAAACATGCCCATGTCTATTTACTTT 146
LILLYCOT-E        TTTTTTAAGTGACGTAAAATCGTGTGAG-GCCAACGACCATAATGCGGGCTGT-TGCCCG 177
PORTICI-N         ACAAAAACAAAAAGGAACCATTTAATAATA-CATAGAAGAAATCTTGAACCACCCATTTG 156
PORTICI-P         ACCCCTCCAATGAGCGGTTGATTAGTCTTGGCGGTTGAGGTTTAAATCACTAGTGAATTC 64
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GHEYSI-D          GCC-TCCACTACCCCGAT---CTCAAACGTCCTTTCGA--GTTCTACGAAAACTACGAGG 201
NADERI-C          GCC-TCCACTACCCCGAT---CTCAAACGTCCTTTCGA--GTTCTACGAAAACTACGAGG 201
Altera-E          GCC-TCCACTACCCCGAT---CTCAAACGTCCTTTCGA--GTTCTACGAAAACTACGAGG 201
KYOTO-B           GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
REALE-D           GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
KYOTO-A           GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
S.CASTRESE-B      GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
LITO-E            GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
BORA-A            GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
S.CASTRESE-A      GCC-TCCACCTCCCAAAT---TCTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
Altera-F          GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
NINFA-A           GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
REALE-E           GCC-TCCACCACCCAAAT---TTTGAGCGTTTGGTCGA--CCCTAATGA----------- 190
BO92639095-B      GCC-TCCACCACCCAAAT---TTTGAACGTCTGGCCGA--CCCTGATGA----------- 190
CORNIA-C          GCC-TCCACCACCCAAAT---TTTGAACGTCTGGCCGA--CCCTGATGA----------- 190
PETRA-B           GCC-TCCACCACCCAAAT---TTTGAACGTCTGGCCGA--CCCTGATGA----------- 190
BO93622312-F      GCC-TCCTCCACCCAAAT---TTTGAACGTCTGGCCGA--CCCTGATGA----------- 190
CORNIA-B          GCC-TCCTCCACCCAAAT---TTTGAACGTCTGGCCGA--CCCTGATGA----------- 190
PETRA-A           GCC-TCCTCCACCCAAAT---TTTGAACGTCTGGCCGA--CCCTGATGA----------- 190
NADERI-B          GCC-TCCACCACCCAAAT---TTTGAACGAAACGACGA--CCCTGATGA----------- 190
NINFA-D           GCC-TCCACCACCCAAAT---TTTGAACGAAACGACGA--CCCTGATGA----------- 190
BORA-B            GCC-TTCACCACCCGAAT---TTTGAATGTGTGGTCGA--CCCTGATGA----------- 190
MAYA-B            GCC-TTCACCACCCGAAT---TTTGAATGTGTGGTCGA--CCCTGATGA----------- 190
PINKCOT-G         GCC-TTCACCACCCGAAT---TTTGAATGTGTGGTCGA--CCCTGATGA----------- 190
PINKCOT-A         GCC-TTCACCACCCGAAT---TTTGAATGTGTGGTCGA--CCCTGATGA----------- 190
MAYA-A            GCC-TTCACCACCCGAAT---TTTGAATGTGTGGTCGA--CCCTGATGA----------- 190
YAMAGATA-C        GCC-TCCACCACCCAAAT---TTTCGACGTCAGAACGA--CAATGATGA----------- 190
YAMAGATA-D        GCC-TCCACCACCCAAAT---TTTCGACGTCAGAACGA--CAATGATGA----------- 190
GHEYSI-A          GCC-TCCACCACCCAAAT---TTTGAACGTCATAACGA--CACTGATGA----------- 190
ROBADA-B          GCC-TCCACCACCCAAAT---TTTGAATGTGTGATCGA--CCCCGATGA----------- 190
ROBADA-D          GCC-TCCACCACCCAAAT---TTTGAATGTGTGATCGA--CCCCGATGA----------- 190
LILLYCOT-A        GCT-TCCACCACCCAAAT---TTTGAATGTTCGGTCGA--CCCTGATGA----------- 189
LILLYCOT-E        GCA-TCCAACGCC--ATT---CATGGCCATATCAATGAT-CTCTGGTGCGTACC-----G 225
PORTICI-N         TCCGTTCAATCACAAAATGCACCCAAATGAATTGAGACC-CAGAAACGAGCAGATTCAGA 215
PORTICI-P         GCG-GCCGCCTGCAGGT------CGACCATATGGGAGAGCTCCCAACGCGTTGGATGCAT 117
                   *    *     *                                  *
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GHEYSI-D          ACTACCCAGATCTTAAACGAGAATTGGAATGGTCACTTTTCTCCAACGAAACATTTGAGC 261
NADERI-C          ACTACCCAGATCTTAAACGAGAATTGGAATGGTCACTTTTCTCCAACGAAACATTTGAGC 261
Altera-E          ACTACCCAGATCTTAAACGAGAATTGGAATGGTCACTTTTCTCCAACGAAACATTTGAGC 261
KYOTO-B           ----CCCATATCCTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
REALE-D           ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
KYOTO-A           ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
S.CASTRESE-B      ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
LITO-E            ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
BORA-A            ----CCCATATCTTAGAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
S.CASTRESE-A      ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
Altera-F          ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
NINFA-A           ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
REALE-E           ----CCCATATCTTAAAAAGGAATTTCAATGGTCTCTTTTTCCCAAAGAAACATTTGAGG 246
BO92639095-B      ----TCCATATGTTAAGCAAGGATTTCAGTGGTCTCTTTTTTCTAATGAAACATTTGAGG 246
CORNIA-C          ----TCCATATGTTAAGCAAGGATTTCAGTGGTCTCTTTTTTCTAATGAAACATTTGAGG 246
PETRA-B           ----TCCATATGTTAAGCAAGGATTTCAGTGGTCTCTTTTTTCTAATGAAACATTTGAGG 246
BO93622312-F      ----CCCATATGTTAAACAAGAGTTTAGCTGGTCTCTTTTTTCGAATGAAACATTTGAGG 246
CORNIA-B          ----CCCATATGTTAAACAAGAGTTTAGCTGGTCTCTTTTTTCGAATGAAACATTTGAGG 246
PETRA-A           ----CCCATATGTTAAACAAGAGTTTAGCTGGTCTCTTTTTTCGAATGAAACATTTGAGG 246
NADERI-B          ----CCCATATGTTGAACAAGAATTTCAATGGTCTCATTTTTCCAATGAAACATTTGAGG 246
NINFA-D           ----CCCATATGTTGAACAAGAATTTCAATGGTCTCTTTTTTCCAATGAAACATTTGAGG 246
BORA-B            ----CCCATATTTGGAAGAAGAGCTTCAATGGTCTCTTTTTTCCAATGAAACATTTAAGC 246
MAYA-B            ----CCCATATTTGGAAGAAGAGCTTCAATGGTCTCTTTTTTCCAATGAAACATTTAAGC 246
PINKCOT-G         ----CCCATATATGGAAGAAGAGCTTCAATGGTCTCTTTTTTCCAATGAAACATTTAAGC 246
PINKCOT-A         ----CCCATATTTGGAAGAAGAGCTTCAATGGTCTCTTTTTTCCAATGAAACATTTAAGC 246
MAYA-A            ----CCCATATTTGGAAGAAGAGCTTCAATGGTCTCTTTTTTCCAATGAAACATTTAAGC 246
YAMAGATA-C        ----CCCATATGATATAGAAGAACTTCAATGGTCGCTTTTCTCTAATGAAACATTTGAGC 246
YAMAGATA-D        ----CCCATATGATATAGAAGAACTTCAATGGTCGCTTTTCTCTAATGAAACATTTGAGC 246
GHEYSI-A          ----CCCATATGACATAGAAGAACTTCAGTGGTCACTTTTTTACAATGAAACGTTCGAGC 246
ROBADA-B          ----CCCATATTTAGAAGAAGAACTTCAATGGTCTCTGTTTTCCAATGTAACATTTGAGA 246
ROBADA-D          ----CCCATATTTAGAAGAAGAACTTCAATGGTCTCTGTTTTCCAATGTAACATTTGAGA 246
LILLYCOT-A        ----TCCAGGTTTTGAACAAGAACTTCAATGGTCTCTTTTTTCATATGAAACATTTGAGC 245
LILLYCOT-E        GGTTGAGAAGCGGTGTAAGTGAACTGCAGTTGCCATGTTTTACGGCAGTGAGAGCAGAGA 285
PORTICI-N         AATTGACAATTTTAATTGAAGAAATTGAAAAGAAAAAAAGGAAGATGAAAAGATTGGAAT 275
PORTICI-P         AGCTTGAGTATTCTATAGTGTCACCTAAATAGCTTGGCGTAATCATGGTCATAGCTGTTT 177
                                                  *                 *
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GHEYSI-D          ATTGCTCCAAGTTAAACCATCCCTTGGGGATCAAGAAAGATT--------------ATAG 307
NADERI-C          ATTGCTCCAAGTTAAACCATCCCTTGGGGATCAAGAAAGATT--------------ATAG 307
Altera-E          ATTGCTCCAAGTTAAACCATCCCTTGGGGATCAAGAAAGATT--------------ATAG 307
KYOTO-B           AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
REALE-D           AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
KYOTO-A           AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
S.CASTRESE-B      AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
LITO-E            AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
BORA-A            AGTACTACAAACTAAGCCATCCCTTAGGGACGACAGAACATT--------------ATGG 292
S.CASTRESE-A      AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
Altera-F          AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
NINFA-A           AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
REALE-E           AGTGCTACAAACTAAGCCATCCCTTAGGGATGACAGAACATT--------------ATGG 292
BO92639095-B      AGTGCTCCAAGTTAAGCCATCCCTTAGGGAGCAGAGAACCTT--------------ATGT 292
CORNIA-C          AGTGCTCCAAGTTAAGCCATCCCTTAGGGAGCAGAGAACCTT--------------ATGT 292
PETRA-B           AGTGCTCCAAGTTAAGCCATCCCTTAGGGAGCAGAGAACCTT--------------ATGT 292
BO93622312-F      AAAGCTCCAAGATAACCCATCCCTTAGGGAGCACAGAACATT--------------ATGG 292
CORNIA-B          AAAGCTCCAAGATAACCCATCCCTTAGGGAGCACAGAACATT--------------ATGG 292
PETRA-A           AAAGCTCCAAGATAACCCATCCCTTAGGGAGCACAGAACATT--------------GTGG 292
NADERI-B          AGTGCTCCAAGTTAAGACATCCCTCAGGGAGCACAGAACATT--------------ATAT 292
NINFA-D           AGTGCTCCAAGTTAAGACATCCCTCAGGGAGCACAGAACATT--------------ATAT 292
BORA-B            TGTGCTCCAAGTTAAGCCATCCCTTAGGGAGTACAAATCGTT--------------ATGG 292
MAYA-B            TGTGCTCCAAGTTAAGCCATCCCTTAGGGAGTACAAATCGTT--------------ATGG 292
PINKCOT-G         TGTGCTCCAAGTTAAGCCATCCCTTAGGGAGTACAAATCGTT--------------ATGG 292
PINKCOT-A         TGTGCTCCAAGTTAAGCCATCCCTTAGGGAGTACAAATCGTT--------------ATGG 292
MAYA-A            TGTGCTCCAAGTTAAGCCATCCCTTAGGGAGTACAGATCGTT--------------ATGG 292
YAMAGATA-C        AGTTCTGCGAATTAAGTCATCCCTTAGGGAGCACGGAACATT--------------ATGG 292
YAMAGATA-D        AGTTCTGCGAATTAAGTCATCCCTTAGGGAGCACGGAACATT--------------ATGG 292
GHEYSI-A          AGTTCTCCAAGTTAAGCCATCCCTTAGGAAGCACAGAACATT--------------ATGG 292
ROBADA-B          AGTGCTCCAAGTTAAGCCATCCCCTAGGAAGCACAAAACATT--------------ATGG 292
ROBADA-D          AGTGCTCCAAGTTAAGCCATCCCCTAGGAAGCACAAAACATT--------------ATGG 292
LILLYCOT-A        AGTGCTCCGAGTTAAGCCATCCCTTCGGGAGCCCAGAATCTT--------------ACCG 291
LILLYCOT-E        TAGCGCTGATGTCCGGCGGTGCTTTTGCCGTTACGCACCACCCCGTCAGTAGCTGAACAG 345
PORTICI-N         TTATATGGGGGAAAAAGTAAAGGGTTGGGAAATCAGTTCACCT----GCATCCCCGCTGG 331
PORTICI-P         CCTGTGTGAAATT--GTTATCCGCTCACAATTCCACACAACATACGAGCCGGAAGCATAA 235
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GHEYSI-D          GGCATATGGCTCA-AGCAATGGCCTAGTTTGC-ATTTCGGATGACAAATTGGACACCAAG 365
NADERI-C          GGTATATGGCTCA-AGCAATGGCCTAGTTTGC-ATTTCGGATGACAAATTGGACACCAAG 365
Altera-E          GGTATATGGCTCA-AGCAATGGCCTAGTTTGC-ATTTCGGATGACAAATTGGACACCAAG 365
KYOTO-B           GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
REALE-D           GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
KYOTO-A           GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
S.CASTRESE-B      GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
LITO-E            GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
BORA-A            GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
S.CASTRESE-A      GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAACTTCGAT 350
Altera-F          GATATATGGCTCC-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
NINFA-A           GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
REALE-E           GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATCCTGAATTTCGAT 350
BO92639095-B      GATTTATGGCTCA-AGCAATGGTTTAGTTTGC-ATTTCCGATGAGATACTGAATTTCGAT 350
CORNIA-C          GATTTATGGCTCA-AGCAATGGTTTAGTTTGC-ATTTCCGATGAGATACTGAATTTCGAT 350
PETRA-B           GATTTATGGCTCA-AGCAATGGTTTAGTTTGC-ATTTCCGATGAGATACTGAATTTCGAT 350
BO93622312-F      GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCAGATGAGATTTTGAATTTCGAT 350
CORNIA-B          GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCAGGTGAGATTTTGAATTTCGAT 350
PETRA-A           GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCAGATGAGATTTTGAATTTCGAT 350
NADERI-B          GATATATGGCTCA-AGCAATGGCTTAGTTTGC-ATTTCGGAGGAGATATTGAATTTCGAT 350
NINFA-D           GATATATGGCTCA-AGCAATGGCTTAGTTTGC-ATTTCGGAGGAGATATTGAATTTCGAT 350
BORA-B            GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATACTGAATTTCGAT 350
MAYA-B            GATATATGGTTCA-AGCAATGGTTTAGTTCGC-ATTTCGGATGAGATACTGAATTTCGAT 350
PINKCOT-G         GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATACTGAATTTCGAT 350
PINKCOT-A         GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATACTGAATTTCGAT 350
MAYA-A            GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATACTGAATTTCGAT 350
YAMAGATA-C        GATATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATACTGAATTTTGAT 350
YAMAGATA-D        GGTATATGGTTCA-AGCAATGGTTTAGTTTGC-ATTTCGGATGAGATACTGAATTTTGAT 350
GHEYSI-A          GGTATATGGTTCA-AGCAATGGTTTAATTTGC-ATTTCGGATGAGATATTGAATTTCGAT 350
ROBADA-B          GATATATGGTTCA-AGCAATGGTTTGCTTTGC-ATTTCTGATGAGATATTGAATTTCGAT 350
ROBADA-D          GATATATGGTTCA-AGCAATGGTTTGCTTTGC-ATTTCTGATGAGATATTGAATCTCGAT 350
LILLYCOT-A        GATATATGGTTCA-ACCAACGGTTTAATTTGC-ATTTCGGATGCGATATTGAATTCGGCG 349
LILLYCOT-E        GAGGGACAGGTGATAGAAACAGAAGCCACTGG-AGCACCTCAAAAACACCATCATACAAT 404
PORTICI-N         GCCAAAGAGAAGGCAATATTGTGTGAAATGGC-ATCCCCGTTGGAG----GTGAGCAAAA 386
PORTICI-P         AGTGTAAAGCCTGGGGTGCCTAATGAGTGAGCTAACTCACATTAATTGCGTTGCGCTCAC 295
                       *  *                     *  *   *
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GHEYSI-D          AGCCC--TATACACATATGGAACCCCTCG-GTTAGGAAATTTA---GAACCCTTCCAATG 419
NADERI-C          AGCCC--TATACACATATGGAACCCCTCG-GTTAGGAAATTTA---GAACCCTTCCAATG 419
Altera-E          AGCCC--TATACACATATGGAACCCCTCG-GTTAGGAAATTTA---GAACCCTTCCAATG 419
KYOTO-B           AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
REALE-D           AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
KYOTO-A           AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
S.CASTRESE-B      AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
LITO-E            AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
BORA-A            AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
S.CASTRESE-A      AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
Altera-F          AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
NINFA-A           AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
REALE-E           AGTCC--TATACACATATGGAACCCATCG-GTTAGGAAATTTA---GGGCCCTTCCAATG 404
BO92639095-B      AGTCC--TATACACATATGGAATCCATCG-GTTAGTAAACTTA---GAACCACGCCAATC 404
CORNIA-C          AGTCC--TATACACATATGGAATCCATCG-GTTAGTAAACTTA---GAACCACGCCAATC 404
PETRA-B           AGTCC--TATACACATATGGAATCCATCG-GTTAGTAAACTTA---GAACCACGCCAATC 404
BO93622312-F      AGTCC--TATACACATATGGAACCCGTCG-GTTAAGAAATTTA---AGACCCCTCCAATG 404
CORNIA-B          AGTCC--TATACACATATGGAACCCGTCG-GTTAAGAAATTTA---AGACCCCTCCAATG 404
PETRA-A           AGTCC--TATACACATATGGAACCCGTCG-GTTAAGAAATTTA---AGACCCCTCCAATG 404
NADERI-B          AGTCC--AATACACATATGGAACCCCTCG-GTTAAGAAATTTA---GGACCCCTCCAATG 404
NINFA-D           AGTCC--AATACACATATGGAACCCCTCG-GTTAAGAAATTTA---GGACCCCTCCAATG 404
BORA-B            AGTCC--TATACACATATGGAACCCATCG-GTAAAGAAATTTA---CGAGCCCTCCAATG 404
MAYA-B            AGTCC--TATACACATATGGAACCCATCG-GTAAAGAAATTTA---CGAGCCCTCCAATG 404
PINKCOT-G         AGTCC--TATACACATATGGAACCCATCG-GTAAAGAAATTTA---CGAGCCCTCCAATG 404
PINKCOT-A         AGTCC--TATACACATATGGAACCCATCG-GTAAAGAAATTTA---CGAGCCCTCCAATG 404
MAYA-A            AGTCC--TATACACATATGGAACCCATCG-GTAAAGAAATTTA---CGAGCCCTCCAATG 404
YAMAGATA-C        AGTCC--TATACATATATGGAACCCATCG-GTTAGGAAATTTA---GGACCCCTCCAATG 404
YAMAGATA-D        AGTCC--TATACATATATGGAACCCATCG-GTTAGGAAATTTA---GGACCCCTCCAATG 404
GHEYSI-A          AGTCC--TATACACATATGGAACCCATCA-ATTAGTAAATTTA---GGACCCCTCCAATG 404
ROBADA-B          AGTCC--AATACACATATGGAACCCATTG-GTTGGAAGATATA---GGACCCCTCCAATG 404
ROBADA-D          AGTCC--AATACACATATGGAACCCATTG-GTTGGAAGATATA---GGACCCCTCCAATG 404
LILLYCOT-A        AGTCC--TATACACATATGGAACCCATCT-GTTAGGAAAGTTATTAGGACCCTCCCAACG 406
LILLYCOT-E        AAATC--AGTAAGTTGGCAGCATCACCTG-GTTGGAAGATATA---GGACCCCTCCAATG 458
PORTICI-N         CTTTC--TTCCCAGGAGAAGTCCCCATTT-TCTAATTGAACTA---GGACTTGACTGAT- 439
PORTICI-P         TGCCCGCTTTCCAGTCGGGAAACCTGTCGTGCCAGCTGCATTA--ATGAATCGGCCAACG 353
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GHEYSI-D          AGCACCAA------CGTTAAA-TTTCGCTACATTGCTCTCCAATTCGGGTTCCACCCC-G 471
NADERI-C          AGCACCAA------CGTTAAA-TTTCGCTACATTGCTCTCCAATTCGGGTTCCACCCC-G 471
Altera-E          AGCACCAA------CGTTAAA-TTTCGCTATATTGCTCTCCAATTCGGGTTCCACCCC-G 471
KYOTO-B           AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
REALE-D           AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
KYOTO-A           AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
S.CASTRESE-B      AGCATCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
LITO-E            AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
BORA-A            AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
S.CASTRESE-A      AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
Altera-F          AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
NINFA-A           AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
REALE-E           AGCACCAACATTAACATTAAA-TTTAGCTGTGTTGCTCTCCAGTTCGGGTTCCACCCT-G 462
BO92639095-B      AGCACCAATATCAGCATTAAA-TTTAGCCATGTTGCTCTCCAATTCGGGTTCCACCCC-A 462
CORNIA-C          AGCACCAATATCAGCATTAAA-TTTAGCCATGTTGCTCTCCAATTCGGGTTCCACCCCCA 463
PETRA-B           AGCACCAATATCAGCATTAAA-TTTAGCCATGTTGCTCTCCAATTCGGGTTCCACCCC-A 462
BO93622312-F      AGCACCAACATTAACATTAAA-TTTAGCCTCGTTTCTCTCCAATTCGGGTTCCATCCT-A 462
CORNIA-B          AGCACCAACATTAACATTAAA-TTTAGCCTCGTTTCTCTCCAATTCGGGTTCCATCCT-A 462
PETRA-A           AGCACCAACATTAACATTAAA-TTTAGCCTCGTTTCTCTCCAATTCGGGTTCCATCCT-A 462
NADERI-B          AGCACCAACATTAACATTAAA-TTTAGCTATGTTGCTCTCCAATTCGGGTTCCACCCC-G 462
NINFA-D           AGCACCAACATTAACATTAAA-TTTAGCTATGTTGCTCTCCAATTCGGGTTCCACCCC-G 462
BORA-B            AGCACCAACATTAACGTCAAA-TTTGCCTATGTTGCTCTCCAATTTGGGTTCCACCCC-A 462
MAYA-B            AGCACCAACATTAACGTCAAA-TTTACCTATGTTGCTCTCCAATTTGGGTTCCACCCC-A 462
PINKCOT-G         AGCACCAACATTAACGTCAAA-TTTACCTATGTTGCTCTCCAATTTGGGTTCCACCCC-A 462
PINKCOT-A         AGCACCAACATTAACGTCAAA-TTTACCTATGTTGCTCTCCAATTTGGGTTCCACCCC-A 462
MAYA-A            AGCACCAACATTAACGTCAAA-TTTACCTGTGTTGCTCTCCAATTTGGGTTCCACCCC-A 462
YAMAGATA-C        AGCACCAACATTAACATGAAA-TTTTCCCATGTTGCTCTCCAATTCGGGTTCCACCCT-G 462
YAMAGATA-D        AGCACCAACATTAACATGAAA-TTTTCCCATGTTGCTCTCCAATTCGGGTTCCACCCT-G 462
GHEYSI-A          AGCACCAACATTAACCTTAAA-TTTGCCTATGTAGCTCTCCAATTCGGGTTCCACCCT-G 462
ROBADA-B          AGCACCAACATTAACATTAAA-TTTAATTATGTTGCTCTCCAATTCGGGTTTCACCCTGG 463
ROBADA-D          AGCACCAACATTAACATTAAAATTTAATTATGTTGCTCTCCAATTCGGGTTTCACCCTGG 464
LILLYCOT-A        AGCACCAACA---ACATTGAA-TTTAGCTACATCGATCTCCACTTCGGGTTCCACCCC-G 461
LILLYCOT-E        AGCACCAACATTAACATTAAA-TTTAATTATGTTGCTCTCCAATTCGGGTTTCACCCT-G 516
PORTICI-N         -ACACTTATAT-AGTGTAAAAACTAAATCAAGAAATGAATTAAAAAAGGTGGGTGGTGTG 497
PORTICI-P         CGCGGGGAGAGGCGGTTTGCGTATTGG--GCGCTCTTCCGCTTCCTCGCTCACTGACTCG 411
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GHEYSI-D          GGGTTAACGACTACAAAGTTGTAA-GGATGTTGCGCGTCCACAAAGATGATGCTTTCGCA 530
NADERI-C          GGGTTAACGACTACAAGGTTGTAA-GGATGTTGCGCGTCCACAAAGATGATGCTTTCGCA 530
Altera-E          GGGTTAACGACTACAAGGTTGTAA-GGATGTTGCGCGTCCACAAAGATGATGCTTTCGCA 530
KYOTO-B           GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
REALE-D           GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
KYOTO-A           GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
S.CASTRESE-B      GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
LITO-E            GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
BORA-A            GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
S.CASTRESE-A      GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCTTTGGCG 518
Altera-F          GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
NINFA-A           GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
REALE-E           GGGTTAATGACTACAGGGCTGTAA-GGATGATGCGTACC---AATAAAAGTGCCTTGGCG 518
BO92639095-B      GGGTTAATGACTTCAAGGCTATAA-GGATGTTGCGTACC---AACAAAAAGGCATTGGCG 518
CORNIA-C          GGGTTAATGACTTCAAGGCTATAA-GGATGTTGCGTACCC--AACAAAAAGGCATGGGCG 520
PETRA-B           GGGTTAATGACTTCAAGGCTATAA-GGATGTTGCGTACC---AACAAAAATGTCTTGGCG 518
BO93622312-F      GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGCACC---AACAAAAATGTCTTGGCG 518
CORNIA-B          GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGCACCC--AACAAAAATGTCTTGGCG 519
PETRA-A           GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGCACC---AACAAAAATGTCTTGGCG 518
NADERI-B          GGGTTAACGACTACAAGGCTGTAA-GGATGATGCGTACC---AACAAAAATGCCTTGGCG 518
NINFA-D           GGGTTAACGACTACAAGGCTGTAA-GGATGATGCGTACC---AACAAAAATGCCTTGGCG 518
BORA-B            GTCTTAATGACTACAAGGTTGTAA-GGATGATGCGTACC---AACAAAGGTGCCTTGGCG 518
MAYA-B            GTCTTAATGACTACAAGGTTGTAA-GGATGATGCGTACC---AACAAAGGTGCCTTGGCG 518
PINKCOT-G         GTCTTAATGACTACAAGGTTGTAA-GGATGATGCGTACC---AACAAAGGTGCCTTGGCG 518
PINKCOT-A         GTCTTAATGACTACAAGGTTGTAA-GGATGATGCGTACC---AACAAAGGTGCCTTGGCG 518
MAYA-A            GTCTTAATGACTACAAGGTTGTAA-GGATGATGCGTACC---AACAAAGGTGCCTTGGCG 518
YAMAGATA-C        GGGTTAATGACTACAAGGTTGTAA-GGATGATGCGTACC---AACAAAGGTGCCTTGGCA 518
YAMAGATA-D        GGGTTAATGACTACAAGGTTGTAA-GGATGATGCGTACC---AACAAAGGTGCCTTGGCA 518
GHEYSI-A          GGGTTAATGACTACAAGGCTGTAA-GAATGATGCGTACC---AACAAAGATGCCTTCGTG 518
ROBADA-B          GG-TTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AACAAAGATGCCTTGGCG 518
ROBADA-D          GGGTTAATGACTACAAGGCTGTAAAGGATGATGCGTACC---AACAAGATGCCCTTGGCG 521
LILLYCOT-A        GAGTTAATGACTACAAGGCCGTAC-GAATGATGCTTATT---GACAAAGATGCCTTCGCG 517
LILLYCOT-E        GGGTTAATGACTACAAGGCTGTAA-GGATGATGCGTACC---AACAAAGATGCCTTGGCG 572
PORTICI-N         TAATAAATAAGAAAAGGG--GAAGTCTGCTCTGCGTCTTGTTCGTGAAAAGAAAAGAGTG 555
PORTICI-P         CTGCGCTCGGTCGTTCGGCTGCGGCGAGCGGTATCAGCTCA-CTCAAAGGCGGTAATACG 470
                                  *             *             *
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GHEYSI-D          GTCGAAGTTTATAGTCTCAGCAGAGACTCTTGG-AAGATGGTT-GAAGAACATCCTCTTT 588
NADERI-C          GTCGAAGTTTATAGTCTCAGCACAGACTCTTGG-AAGATGGTT-GAAGAACATCCTCTTT 588
Altera-E          GTCGAAGTTTATAGTCTCAGCACAGACTCTTGG-AAGATGGTT-GAAGAACATCCTCTTT 588
KYOTO-B           GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCTT 576
REALE-D           GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCTT 576
KYOTO-A           GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCTT 576
S.CASTRESE-B      GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCTT 576
LITO-E            GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCTT 576
BORA-A            GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCTT 576
S.CASTRESE-A      GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCTT 576
Altera-F          GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCAATTCCTCCCT 576
NINFA-A           GTTGAGGTTTATAGTCTCAAAAGAGACTC-TTGGAAGATGATT-GAAGCATCTCCTCCTT 576
REALE-E           GTTGAGGTTTATAGTCTCAAAAGAGACTCCTTGGAAGATGATT-GAAGCAATTCCTCCTT 577
BO92639095-B      GTTGAGGTTTATAGTCTCAGAGCAGACTCCTGG-AAGATGATT-GAAGCAATTCCTCCTT 576
CORNIA-C          GTTGAGGTTTATAGTCTCACACCAAACTCCTGC-AATATGATC-GC-GCCACTCCTCCCT 577
PETRA-B           GTTGAGGTTCATAGTCTCAGCACAGACTCTTGG-AAAATGGTT-GAAGCAATTCCTCCTT 576
BO93622312-F      GTTGAGGTTTATAGTCTCAGCACAGACTCTTGG-AAAATGGTT-GAAGCAATTCCTCCTT 576
CORNIA-B          GTTGAG-TTTATAGTCTCAGCACAGACTCTTGGCAAAATGGTTTGAAGCAATTCCTCCTT 578
PETRA-A           GTTGAGGTTTATAGTCTCAGCACAGACTCTTGG-AAAATGGTT-GAAGCAATTCCTCCTT 576
NADERI-B          GTTGAGGTTTATAGTCTCAAAACAGACTCTTGG-AAGATGATT-GAAGCAATTCCCCCGT 576
NINFA-D           GTTGAGGTTTATAGTCTCAAAACAGACTCTTGG-AAGATGATT-GAAGCAATTCCCCCGT 576
BORA-B            GTTGAGGTTTATACTCTCAGAACGGACTCTTGG-AAGATGATT-GAAGCAATTCCTCCTT 576
MAYA-B            GTTGAGGTTTATACTCTCAGAACGGACTCTTGG-AAGATGATT-GAAGCAATTCCTCCTT 576
PINKCOT-G         GTTGAGGTTTATACTCTCAGAACGGACTCTTGG-AAGATGATT-GAAGCAATTCCTCCTT 576
PINKCOT-A         GTTGACGTTTATACTCTCAGACCGGACTCCTGG-AAGATGATT-GAAGCAATTCCTCCTT 576
MAYA-A            GTTGAGGTTTATACTCTCAGAA-GGACTCTTGG-AAGATGATT-GCAGCACTTCCTCCTT 575
YAMAGATA-C        GTTGAGGTATATAGTCTTAGAACAGACTGTTGG-AAAATGATT-GAAACAATTCCTCCTT 576
YAMAGATA-D        GTTGAGGTATATAGTCTTAGAACAGACTGTTGG-AAAATGATT-GAAACAATTCCTCCTT 576
GHEYSI-A          GTTGAG-TTTATAGTCTTCAAACAGACTCTTGG-AAGATGATT-GAAGCAATTCCTCCTT 575
ROBADA-B          GTTGAGGTTTATAGTCTTAGAACAGACTCGTGGAAGATGATTGA--AGCAATTCCTCCTT 576
ROBADA-D          GTTGCAGTTTATACTCATACACCC-ACTCACGACAGATGCGTCACCAACAATCTCCCTCG 580
LILLYCOT-A        GTTGAGGTTTATAGTCTTACCACTGATTCTTGG-AAGCTGATT-GAAGTAATTCCTCCTT 575
LILLYCOT-E        GTTGAGGTTTATAGTCTTAGAACAGACTCGTGG-AAGATGATT-GAAGCAATTCCTCCTT 630
PORTICI-N         AACGAG--TCAGTCTCTTCTCTCTGACTA-----AAGATTAAATATAGCTTTCTTTCGTT 608
PORTICI-P         GTTA---TCCACAGAATCAGGGGATAACGCAGGAAAGAACATG-TGAGCAAAAGGCCAGC 526
                            *              *       *                     *
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GHEYSI-D          GGTTAA----AATGCACTTGGCAG-ACCACAGGGG-TACATTTTAT-AA-TGGAGTAGCA 640
NADERI-C          GGTTAA----AATGCACTTGGCAG-ACCACAGGGG-TACATTTTAT-AA-TGGAGTAACA 640
Altera-E          GGTTAA----AATGCACTTGGCAG-ACCACAGGGG-TACATTTTAT-AA-TGGAGTAGCA 640
KYOTO-B           GGTTAA----AATGCACTTTGGAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTAGCA 629
REALE-D           GGTTAA----AATGCACTTTGGAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTAGCA 629
KYOTO-A           GGTTAA----AATGCACTTTGGAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTAGCA 629
S.CASTRESE-B      GGTTAA----AATGCACTTTGGAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTAGCA 629
LITO-E            GGTTAA----AATGCACTTTGGAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTAGCA 629
BORA-A            GGTTAA----AATGCACTTTGGAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTAGCA 629
S.CASTRESE-A      GGTTAA----AATGCACTTTGGAACATCATAGGG--TACGTTTTTC-AG-TGGAGTAGCA 628
Altera-F          GGTTAA----AATGCACTTTGCAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTACCA 629
NINFA-A           GGTTAA----AATGCACTTTGGAACATCATAGGGG-TACGTTTTTC-AG-TGGAGTAGCA 629
REALE-E           GGTTAAC---AATGCACTTTGGAACATCATATGGG-TACGTTTTTC-AA-CGGATTACCA 631
BO92639095-B      GGTTAA----AATGCACTTGGCAGCATCATGACGG-CACATTCTTT-AA-TGGAGTAGCA 629
CORNIA-C          GGCCTA----CACGCCACTTGCCCCATCCTCCCCG-CCCACCCTTT-AA-TGGACTAACT 630
PETRA-B           GGTTAA----AATGCACTTGGCAGCATCATAACGGGTACATTTTTTTAA-TGGAGTAGCA 631
BO93622312-F      GGTTAA----AATGCACTTGGCAGCATCATAACGGGTACATTT-TTTAG-TGGAGTAGCA 630
CORNIA-B          GGTTAA----AATGCACTTCGCAGCATCATCACGGGTACATTTCTTTAC-TGCAGTAGCA 633
PETRA-A           GGTTAA----AATGCACTTGGCAGCATCATAACGG-TACATTT-TTTAA-TGGAGTAGCA 629
NADERI-B          GGTTAA----AATGTACTTGGCAGCATCATAACGG-TACATTTTTT-AA-TGGAGTAGCA 629
NINFA-D           GGTTAA----AATGTACTTGGCAGCATCATAAAGGGTACATTTTTT-AA-TGGAGTAGCA 630
BORA-B            GGTTAA----AATGCACTTGGCAGCATCATAAAGGGTATGTTTTTTAAA--GGAGTAGCA 630
MAYA-B            GGTTAA----AATGCACTTGGCAGCATCATAA-GGGTATGTTTTTTAAA--GGAGTAGCA 629
PINKCOT-G         GGTTAA----AATGCACTTGGCAGCATCATAA-GGGTATGTTTTTTAAA--GGAGTAGCA 629
PINKCOT-A         GGTTAA----AATGCACTTGGCAGCATCATAA-TGGTATGTTTTCTTAAACGGAGCACCC 631
MAYA-A            GGTTAA----AATGCACTTGGCAGCATCATAA-CGGTATGTTTTTTTAA--GGAGTA-CA 627
YAMAGATA-C        GGTTAA----AATGCACTTGGCAACATCATAAGGG-CAAATTTTTT-AA-TGGAGTAGCA 629
YAMAGATA-D        GGTTAA----AATGCACTTGGCAACATCATAAGGG-CAAATTTTTT-AA-TGGAGTAGCA 629
GHEYSI-A          GGTTAA----AATGCACTTGGAGACATCATCAAGG-TACATTTTTT-AA-CGGAGTAGCA 628
ROBADA-B          GGTTAA----AATGCACTTGGCAGCATCATATGGG-TACATTTTTTAATGGAGTAGCATA 631
ROBADA-D          GCCCCA----AATGCCCCACAC------------------------------------- 598
LILLYCOT-A        GGTTAA----AATGCACTTGGCAGCATCATATGGG-TACATTTTTT-AA-TGGAGTAGCA 628
LILLYCOT-E        GGTTAA----AATGCACTTGGCAGCATCATATGGG-TACATTTTTT-AA-TGGAGTAGCA 683
PORTICI-N         TGGGAATTGAGATGCAGAGGTAGAGATTACACACG-CAGAGCTTGCCAA--ATATCAATT 665
PORTICI-P         AAAAGGCC--AGGAACCGTAAAAAGGCCGCGTTGCTGGCGTTTTTCCATAGGCTCCGCCC 584
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GHEYSI-D          TACC-ACATTATT--GAGAAATTTCCTCTAT-TCAGCG-TT-ATGTCATT-CGATTCAGG 693
NADERI-C          TACT-ACATTATT--GAGAAATTTCCTCTAT-TCAGCG-TTTATGTCTTT---------- 685
Altera-E          TACC-ACATTATT--GAGAAATTTCCTCTAT-TCAGCG-TT-ATGTCATT-CGATTCAGG 693
KYOTO-B           TACC-ACATCATT--CAGAA-CGTCCT-ATGCTCAGCCATTTATGTCAT-TCGATTCAGG 683
REALE-D           TACC-ACATCATT--CAGAA-GGTCCT-ATGCTCAGCA--TTATGTCAT-TCGATTCAGG 681
KYOTO-A           TACC-ACATCATT--CAGAAAGGTCCT-ATGCTCAGCA--TTATGTCAT-TCGATTCAGG 682
S.CASTRESE-B      TACC-ACATCATT--CAGAAAGGTCCT-ATGCTCAGCA--TTATGTCAT-TCGATTCAGG 682
LITO-E            TACC-ACATCATT--CAGAAAGGTCCT-ATGCTCAGCA--TTATGTCAT-TCGATTCAGG 682
BORA-A            TACC-ACATCATT--CAGAAAGGTCCT-ATGCTCAGCA--TTATGTCAT-TCGATTCAGG 682
S.CASTRESE-A      TACC-ACATCATT--CAGAAAGGTCCT-ATGCTCAGCA--TTATGTCAT-TCGATTCAGG 681
Altera-F          TACC-ACATCATTTCCCAATAGGTCCT-ACGCTCCGCA--CTATGTCCTCTCATTTC-GG 684
NINFA-A           TACC-ACATCATT--CCGACAGGTCCTTAAGCTCACCA--TTATGTCCTTTCGATTCAGG 684
REALE-E           TACCCACATCATT--CAGAAAGGTCCT-ATGCTCAGCA--CTATGTCAT-TCGATTCAGG 685
BO92639095-B      TACC-ACATCATT--GAAAAAGGTCCTATAT-TCAGTA--TTATATCCTT-CGATTCAGG 682
CORNIA-C          ACCC-TTACCTCA--GATTACCGCCCGACCCATCATTA--TTATATACTT-CTATTCACG 684
PETRA-B           TACC-ACATCATT--CAGAAAGGTCCTCTAT-TCAGCA--TTATGTCCTTTCGATTCAGG 685
BO93622312-F      TACC-GCATCATT--CAGAAAGGTCCTCTAT-TCAGCA--TTATGTCCTT-CGATTCAGG 683
CORNIA-B          TACC-ACATCATT--CAGAACGGTC-TCTAT-TCATCA--T-ATGTC--T-CGATTCAGG 682
PETRA-A           TACC-ACATCATT--CAGAA-GGTCCTCTAT-TCAGCA--TTATGTCCTT-CGATTCAGG 681
NADERI-B          TACC-ACGTCCTT--CTGAAAGGTCCTATAT-TCAGCA--TTATGTCATTTCGATTCAGG 683
NINFA-D           TACC-ACGTCCTT--CTGAAAGGTCCTATAT-TCAGCA--TTATGTCATT-CGATTCAGG 683
BORA-B            TACAGC-ATCATTG-A-GAAAGGTCCTATGT-TCAGCA--TTATGTCATTC-GATTCAGG 683
MAYA-B            TACAGC-ATCATTG-A-GAAAGGTCCTATGT-TCAGCA--TTATGTCATTC-GATTCAGG 682
PINKCOT-G         TACAGC-ATCATTG-A-GAAAGGTCCTATGT-TCAGCA--TTATGTCATTC-GATTCAGG 682
PINKCOT-A         TACCCCCATCATTG-ACGAAATGTCCTATGT-TCCCCA--TTATGTCACTCCGATTCAGG 687
MAYA-A            TACAGC-ATCATTG-AAAAACGGTCCTATGT-TCAGCA--TTATGTCATTC-GATTCAGG 681
YAMAGATA-C        TACC-ACGTCATT--AAGAAAGGTCCTATAT-TCAGCA--TTATGTCCTT-CGATTCAGG 682
YAMAGATA-D        TACC-ACGTCATT--AAGAAAGGTCCTATAT-TCAGCA--TTATGTCCTT-CGATTCAGG 682
GHEYSI-A          TATC-ACATCATC--GAGAAAGGTC-TCTAT-TCAGCA--TTATGTCATT-CGATTCAGG 680
ROBADA-B          CCATATTATTGAGAAAGGTCCCATATTCAGCATTATGTCCTTCGATTCAGGCAGTGAAGA 691
ROBADA-D          ------------------------------------------------------------
LILLYCOT-A        TACC-ATATTATT--GAGAAAGGTCCCATAT-TCAGCA--TTATGTCCTT-CGATTCAGG 681
LILLYCOT-E        TACC-ATATTATT--GAGAAAGGTCCCATAT-TCAGCA--TTATGTCCTT-CGATTCAGG 736
PORTICI-N         TGAAAACGTAATT--CTGATTCATTCATGAC-TCATTAGGCTTCTGCGCGCGTCTGCACA 722
PORTICI-P         CCCTGACGAGC---------------------------------------------- 595
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GHEYSI-D          CAGTGAAGAAAT-------------------------------------------------- 705
NADERI-C          ----------------------------------------------------------
Altera-E          CAGTGAAGAAAT-------------------------------------------------- 705
KYOTO-B           CAGTGAAGAAAT-------------------------------------------------- 695
REALE-D           CAGTGAAGAAAT-------------------------------------------------- 693
KYOTO-A           CAGTGAAGAAAT-------------------------------------------------- 694
S.CASTRESE-B      CAGTGAAGAAAT-------------------------------------------------- 694
LITO-E            CAGTGAAGAAAT-------------------------------------------------- 694
BORA-A            CAGTGAAGAAAT-------------------------------------------------- 694
S.CASTRESE-A      CAGTGAAGAAAT-------------------------------------------------- 693
Altera-F          CATTGAATAACA-------------------------------------------------- 696
NINFA-A           CAGTGAAGAAAT-------------------------------------------------- 696
REALE-E           CAGTGAAGAAAT-------------------------------------------------- 697
BO92639095-B      CAGTGAAGAAAT-------------------------------------------------- 694
CORNIA-C          CTCCCACGCACACACCCCTACACTCACATCCGACCCCACCCAGTCCGCTCACCCCCAATC 744
PETRA-B           CAGTGAAGAAAT-------------------------------------------------- 697
BO93622312-F      CAGTGAAGAAAT-------------------------------------------------- 695
CORNIA-B          CAGTGAAGAAAT-------------------------------------------------- 694
PETRA-A           CAGTGAAGAAAT-------------------------------------------------- 693
NADERI-B          CAGTGAAGAAAT-------------------------------------------------- 695
NINFA-D           CAGTGAAGAAAT-------------------------------------------------- 695
BORA-B            CAGTGAAGAAAT-------------------------------------------------- 695
MAYA-B            CAGTGAAGAAAT-------------------------------------------------- 694
PINKCOT-G         CAGTGAAGAAAT-------------------------------------------------- 694
PINKCOT-A         CAGTGAAGAAAT-------------------------------------------------- 699
MAYA-A            CAGTGAAGAAAT-------------------------------------------------- 693
YAMAGATA-C        CAGTGAAGAAAT-------------------------------------------------- 694
YAMAGATA-D        CAGTGAAGAAAT-------------------------------------------------- 694
GHEYSI-A          CAGTGAAGAAAT-------------------------------------------------- 692
ROBADA-B          AAT----------------------------------------------------------- 694
ROBADA-D          ----------------------------------------------------------
LILLYCOT-A        CAGTGAAGAAAT-------------------------------------------------- 693
LILLYCOT-E        CAGTGAAGAAAT-------------------------------------------------- 748
PORTICI-N         CATTTCATGGGTTTCACG-------------------------------------------- 740
PORTICI-P         ----------------------------------------------------------
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The End