

AMPLIFICATION AND SEQUENCING OF *Rosaceae* EXPRESSED SEQUENCE TAGS (ESTs) AS A RESOURCE FOR FUNCTIONAL GENOMICS DATABASES

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Rosaceae genome size is small (300 Mb) of about twice that of *Arabidopsis* (Baird *et al.*, 1994). Rosaceae members are characterized by a relatively short juvenile period (2-3 yrs) and extensive genetics and genomics resources such as molecular marker maps, interesting mutants and clone library resources (Georgi *et al.*, 2002). In addition, it has been demonstrated that molecular marker tools developed in peach are easily applied to other species in the family (Joobeur *et al.*, 1998; Zhebentyayeva *et al.*, 2003). Peach (*Prunus persica*) is being developed as a model organism for *Rosaceae*, an economically important family that includes fruits and ornamental plants such as apple, pear, strawberry, cherry, almond and rose. To demonstrate the utility of the integrated and fully annotated database and analysis tools, they described a case study where they anchored *Rosaceae* sequences to the peach physical and genetic map by sequence similarity (Jung *et al.*, 2004).

Several marker maps of *Prunus* fruit crops have been published, three of

them, using peach (Rajapakse *et al.*, 1995), almond x peach (Foolad *et al.*, 1995) and almond (Viruel *et al.*, 1995) progenies, were constructed mainly with RFLP markers. Joobeur *et al.* (1998) found that the Texa x Earlygold map has a level of saturation similar to these maps, and therefore it covers most of the distance of the *Prunus* genome and has a sufficient marker density for use in plant breeding. However, its total distance (491 cM) is clearly shorter when compared to the potato (684 cM), tomato (1276 cM) and rice (1491 cM) maps. This difference may be due either to the small nuclear DNA content of the *Prunus* genome, about two and four times smaller than the rice and tomato genomes, respectively, (Arumuganathan and Earle, 1992).

Expressed sequence tags (ESTs) are considered as a functional genomic resource in plant molecular biology. It is produced by transcriptome (the transcribed portion of the genome) sequencing. EST was analyzed in many plant species i.e., in *Arabidopsis* (Spiegelman *et al.*, 2000), in grapes (Scott *et al.*, 2000), *Pinus radiata* and *Pinus taeda* (Cato *et*

al., 2001), sugar beet (Schneider *et al.*, 2002), rice (Jin *et al.*, 2003), in *Ginkgo biloba* (Brenner *et al.*, 2005) and in tomato (Labate and Baldo, 2005). Annotations of ESTs include contig assembly, putative function, simple sequence repeats, and anchored position to the peach physical map where applicable. The importance of high-quality fruit and the intrinsic difficulties of breeding in a perennial species require the development and application of structural and functional genomic databases for the sustained improvement of rosaceous fruit crops. Identification and characterization of genes controlling the genetic basis of the traits, and their tagging with molecular markers, permits facilitated introgression of important characters, speeding development of new breeding material combining the best traits formerly isolated in separate varieties (Abbott *et al.*, 2006).

The ESTree db (Lazzari *et al.*, 2004, 2005, 2007, 2008) is Expressed Sequence Tags (ESTs) database that was developed by the Italian ESTree Interuniversity Centre as a platform for easy genomics and functional genomics data integration and retrieval. Together with the GDR database (genome database for *Rosaceae*), it represents the most complete online resource for peach EST analysis. The ESTree db sequence analysis is based on a semi-automated Perl pipeline that during its steps feeds the tables of a MySQL database. Queries to the database can be performed via a PHP-based web interface. The ESTree and the

GDR databases represent the only existing online resources dedicated to peach EST analysis. The two databases are very similar in terms of entry number (71,540 peach sequences in the ESTree db, 70,939 in the GDR db), but quite different in terms of information and its retrieval. The ESTree db clustering procedure produced a dataset of 27,097 unigenes, 4,303 of which were derived from our in-house prepared libraries (Lazzari *et al.*, 2008).

The aim of this study was to amplify, isolate and sequence some peach, almond and their F₁ progeny ESTs for GDR and ESTree databases as functional genomic resources.

MATERIALS AND METHODS

Earlygold peach cultivar DNA, Texas almond cultivar DNA and F₁ of cv Texas (almond) x cv Earlygold (peach) DNA and 60 ESTs primers (30 forward and 30 reverse primers as in Table (1) were obtained from Parco Tecnologico Padano, Lodi, Italy as a partial contribution in the ESTree project.

ESTs amplification

50 µl PCR reaction were prepared as follows: 45 µl PCR super mix (Invitrogen), 1 µl forward primer, 1 µl reverse primer and 3 µl DNA (20 ng/µl).

PCR cycle profile

Primary denature at 95°C for 3 min one cycle and 35 cycles with the

following profile 95°C for 45 sec, 57°C for 1 min and 68°C for 1 min, and a final extension at 68°C for 5 min.

Two µl of the PCR product were then visualized on ethidium bromide stained 1.5% agarose gel and photographed (Examples in Fig. 1).

DNA sequencing

The PCR product was purified, quantified (and adjusted as 10 ng/ µl) and automatically sequenced using Applied Biosystem Prism 377 Semiadaptive Version 3.2 DNA sequencer (Perkin-Elmer).

EST sequence analysis

The resulted sequences were aligned to NCBI sequences to look for similarities using blastn.

RESULTS

Thirty successful EST sequences were obtained from PCR amplification using 60 EST specific primers. Figure (1) shows some amplified ESTs.

The first EST resulted from the amplification of Texas almond cultivar DNA using primer no. A7 that annotates to farnesylated protein (ATFP6) is 659 bp. The resulted sequence of this EST is as follows:

```
TGATGCTCGGGGTGGGAGTTCAGGCTCAGGA
ACGCAAGCAATCCAGGTATTTTTTTCCTT
CACAAAATCTCCATATCCAAAAGGGCTTAT
AAAAAGTAAATCAAGAAAATCCAAAGTGATT
```

```
TTTAAATTTTTTTTTTTTTTGGGGCGGAAAA
AAAGAATTTTTTTTACTTGTTTTTGTGGA
GGTTTTTTTTTTTTTATTCTTGGAATTTCCA
AAAGGGGAGGCAAGGGATCCCAACACCCCAA
AAGTTGTGGGCTTTTTTTTTTTTTTAAAAA
AAAACTTTTTTTATTCTTTTTAGCTGGGGG
GGGGGCTCCTCTTTTTTTTTTTTCTTTAGC
GGGTAGCTCCCCCAAACCCCAAAAAAAA
ACAACGGGGGGGTGCCGCTGTACAAAACC
AACTAAACAACAATACTTCTTTGCCGACATT
TTGGTTTTTTTTTCCCACGGGGGAAACCCGG
AGAAAAAGGCCGCTAAGAACGAGAGAGGGG
GAAGTAAAACCTGGTGAAAGGGGAAAGAAGG
GGACGCCAGTGAGGGCGGAGAAAAGGCCCCC
CATACACCTTCCCCGGCAATTAGGACCCCC
GGGGGGGGGGGGGGGGCGTTGTCCCCCTCCC
AGAGGGGGAGAAAAGGGGGGGTTTTCGCGCC
GCAAAAAA.
```

The NCBI BLAST search of this EST showed no significant similarity.

The second EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A12 that annotates to phosphorybosyl anthranilate transferase 1 (*Arabidopsis thaliana*) is 241 bp. The resulted sequence of this EST is as follows:

```
TCCATTACCGTTGGGAGGACTCCTACAATGG
CTCTCGATCATGATGAGTTCAAAACGTGATAT
CTGAGGCATCCTTAATAAAACAATTGGATCTT
TCTTCATTGTCTGAAATCATTAATCAAACG
GATTAAGAATTGATAATTTTTATCACAAC
CTGAAGATGTTAATTATATATGGGGGACCAC
TCACATTGGATATGTCTACCCCTTGATCAAG
GGGGCTGATAGCCTTCCCTGAAAAA.
```

The NCBI blast search of this EST showed no significant similarity.

The third EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A16 that annotates

to isoflavone reductase related protein (*Pyrus communis*) is 322 bp and showed the following sequence:

```
CGTCCGCCTCAGTTGGCTGACAAGGTACGAT
CATCGCTGCTATCAAGGAAGCTGGCAATGTT
AAGGTAAAAGTTTCGATCTTTTATCTTGTTT
TTCTCTGTTTTTGCCTTCTTGTGTTTCGTGTTT
TCTGTGTTGCGATCGTGAATGTGTCGTTGGG
TTTCAGAGGTTTTTCCCATCTGAGTTTGGAA
ACGACGTGGATCGAGTTCATGCTGTTGAGCC
AGCAAAAAGTGCATTTGCAACCAAGGCCAAA
ATTTCGAGAACGATTGAGGCTGAGGGGATCC
CTCACACCTATGTGGCCTCCAACCTCTTTGC
TGCGACAATAT .
```

The NCBI BLAST search of this EST showed no significant similarity.

The fourth EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A20 that annotates to cinnamyl-alcohol dehydrogenase, putative (CAD) (*Arabidopsis thaliana*) is 695 and showed the following sequence:

```
TCTTTTTGGTGTGTAACAGGAGCATCTGGTT
TCATAGCATCCGGCTGGTGAAGCTCTTACT
GGAACGAGCTTATATGTCAAAGCAACCGT
CCGTGACCCAAGTCAGTGTATTTATATAGAT
ATGCCACTACTATTTAATCTCTTCCCTTT
TTTCTCTCTCCGAAAAAGGTTTTCTTTG
GAGATTTTCATTTTCAACTTCTTGTTTTGA
TGACATCAACAGAAATTTCTGGATTTCAATT
ATTGTTCTCATCCACGATCATATCGAAATAA
CACTTTTCTTCAGATCCAGCCTTATCATCAT
GTGCTAAAAATATGTCAAAGTAGATTTGGA
CCCAACACCATGAGGAGAGAGGAGAGGAG
AAGACAGAGTGAATATTAAGAGGAAAACAA
TTGTGTCAAAGTGAATGAGAGTTGTA
TCTCCCTAACATATAACTAACTCTCTAACTG
TATTCATAACAATACAATTAGTGACTAATC
TTGTACGGTGAGACCTTTTTTTATGAACTAA
TTACTTTTGTAAATATGCATATGCCACAGAT
GACCCGAAGAAAAAGAACTTATTGGCAC
```

```
TTGAGGGGGCAAAGAAAGGCTCCATTTGTT
CAAAGCAGATTTGTTAGAAAGGATCTTTT
GATGCTGTTGTGATGGATGTGAGGGTGTTTT
CATACAAGCTTCC .
```

The NCBI blast search of this EST showed no significant similarity.

The fifth EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A21 that annotates to putative cinnamoyl-CoA reductase (*Oryza sativa*, japonica group) is 676 bp and showed the following sequence:

```
CCCCCCTTACAAGAGGGGGGGGGGGCGCCG
CCCGTTTTTTCATATGGAGGAAGGAGTCAT
CGACTCCCCTTAATTTTTTGTGCGGGGGGA
TGATGGGCCTTTGGAACCATAAGATCACCCC
TTCCAAAAAATTGGGGTCTGAATTTGGGTGG
GGAAAAAAGGGGACAACATACAGTGAAAAAT
TTTGATTTGTGGCAACAAAGTATTTGGTTTG
TGCCATTTCTCTTTTCAGAAAGAACTCGCA
CTTTTTTCGTTTCTACATTTAAGGAGAGAAA
AAAATTGTCTTTTTTTGCCCGTGTGAGAAAT
GGGATTTGAACCTCTCTTTTTTTCTTATCCC
AACCACCATAATGTTTTACAATGTAATAAGT
CCCTTACATCCCTTCCAGAAAAAATTACGG
GATTAATATTTGTTGAAGAACACAGTCTTTT
AGTCAAGAAAGCCAATAACGCACAGGGGTTT
TATTTCTTTAAAGTAATAATTAATTTTTTTT
TTTTCCATTAGGATTTTAAAGATATAAATTAA
GCTCCGCCACTGTTATCAGCCGACTTTCTCA
TGTTTTTGGTAGCCCAATTTGGGTTTTGGACC
TAAAAAAAAGATAAAAATAACCACCTTTCTT
CCCGAAAAAACCTCCACTATTAGGCAAATA
TTAAATTAAACAAGGTGTAATACCA
```

The NCBI BLAST search of this EST showed no significant similarity.

The sixth EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A22 that annotates to cinnamic acid 4-hydroxylase

(*Lithospermum erythrorhizon*) is 161 bp and showed the following sequence:

```
CCTCTTTGGGGGGCTCTTTCCCTTCAAAAAC
ATCTCTCGGCGTTAACTCCTCCGGCGTCCTC
TAGAAGGCAGGGGTGTTTTTGGGCAAATGCA
TCAAAAAGGATTTTTTCTCCTCCGGAATGGG
GAGCTGCCAGCTTTTCTTTTTTTTATGTCTTT
TGGGGAAAAAAAATAT.
```

The NCBI BLAST search of this EST showed no significant similarity.

The seventh EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A23 that annotates to putative cinnamoyl-CoA reductase (*Prunus avium*) is 783 bp and showed the following sequence:

```
TTTTTTTTTTTTTTTTCTCCCCGACCGCCCC
GGGGTGGCGTTCCTTCTCGCCACACCGCCAC
AATAAAAACAATATTTCTTGGGCGAAGCTAT
TTTCCAACCTTATGTTCCCGAAGCTTGACCC
GTAAAAGGGGGTCAAGTAAAGATAAAAAGCAG
TACCAAGGTAGCATTTTGATCTTCTTTACAA
TTTATTTTAGTAAAAATACCTTCTTTTTTTT
TCCTTATTTTGGGAGTAGGCCTTTCCTTTTT
TTTTAGTAAGCCTTACCAATCCGTTACAATC
TCTTCCCCTCAACTCACCCGACCTATTTAA
TTAAATCCAATCCCTTCGACCTTTGAAGAG
AACACCATTTCAAGTCCATTGACCTGGAGCTG
TGTTGAGATGAGGTGAGCTATATCTTGTGT
TGTTTTGCTTTCCATCATAACGTAGGGGATC
AGCAACAAACACTTCAGATTTGACGTTGTGA
AGCTTATTGTTGTGGACACGAAACCCAACTC
CTAAACTTAAGATATGCACATAGTTAAGCCA
AAAAAATATATATGCACATAGTTATGGAAA
AATATATATATGCACATAGATTCAAGTTGCAA
AACCACTTTCCAGGTGTCTAAGGCCTGGAAT
TTACCTCGGCACCTGTACTCAGGGTAAAGT
TCAGCTACCTTGCCACGAAGTCCCATAAT
GTGATATAGCTTCAACGCACAGGTGTCTACC
AGTGGCCGATTTGTTCTCATACACTAAAATG
```

```
TGTGCAAGAGCTACATCTTTAAAATCCACCG
GCCCTCAT.
```

The NCBI BLAST search of this EST showed 19% similarity with *Prunus avium* putative cinnamoyl-CoA reductase (CCR) mRNA.

The 8th EST resulted from the amplification of Texas almond cultivar DNA using primer no. A24 that annotates to ripening-induced protein (*Fragaria vesca*) is 453 bp and showed the following sequence:

```
TTTTGAACTGGTCCCCTTAGTCCCAAATACA
TATGCTCCTGCAATAGCCAAAAATTCATTAG
TGACTTGTGAAAATTGGTCAAAAACAAAATA
CTTAGCTGATAACTGAGACACTAGGAATGTT
ACCAAGTGTAGGCATCAAAAAGGAGAGCACA
GCAATTCAGCAATCTTTAATGGCAACCCGG
TTTTGATCATATCTTGGATTTCATGTGGCC
AGTGGTAAATCCAACCTACATTTGAAGGTGTT
CCTGTTGGAAGCAAGAAAGAAAATTGTGCC
CAATGGCTCCAGGAACCATAAGGAGGAGTGG
ATTTACATGCATGATTTTGGCTATTTGAATT
AGAAGAGGCACAACCAGCGTGGTGGTGGAGT
TGTTTGTATGAAACTCAGTGATGGTGTACT
TATGAGACAGACGGCAGGCGCAATGGCAAAA
TATGGAAGTGCCTCCCCA.
```

The NCBI BLAST search of this EST showed 98% similarity with *Prunus persica* (peach) BAC clone 82I18, complete sequence, 67% similarity with *Vitis vinifera* contig VV78X109361.11, whole genome shotgun sequence and 66% with PREDICTED: *Vitis vinifera* hypothetical protein LOC100255398 (LOC100255398), mRNA.

The 9th EST resulted from the amplification of Texas almond cultivar DNA using primer no. A25 that annotates

to ripening regulated protein DDTR10 (*Lycopersicon esculentum*) is 272 bp and showed the following sequence:

```
TGGGAAGGATGCTCATTTCGTTTCATGCAGCT
CTTTCAAAGCCTCCATCATCAGAATTTGTGA
ACGTGTCTCGGTGGTACAACCATATCACTGC
ACTTCTTAGGATTCGTAAGTTCTTATAACT
TGTGATGGGTTTTCTTTATCTTTATTATTAG
ATTATGATGGATTTCTTCAAAGCTCATATAT
TTGGGTTAAATCTTTGACTTATTTGATTATC
TCTGGTGGTGTAGGGGTGTTTCTGGACAAGG
CTCTGGTGTCTTTGTTGAAGAAAA.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 10th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A27 that annotates to diphenol oxidase is 312 bp and showed the following sequence:

```
TTTGGATAAAACGCCCGGATTTGCGTAGTG
ATTTTGATGCAGCCGTTCCGTCGGCGAAATC
AGGAAGAATCGGAGTGGGAATGGTTGAGGGG
GTGGTGCTGCCGTTGTATTGAAGAATGGCAG
AGGTGGTGCTGTTGTTGAATGCAACATCCCC
ATCAACAAAAGGATGGGAAGCTACGTGATAG
TGCTGGGAGACTGGTTTGCAACTACCAAAA
TGTCATGGTTTTGGCCTGGAGTTATCATGAG
GTAGGAGGTGGTTATAGTTTTATGTATGCA
CCATCTTGAGCTACCACTGTGATGTTGTGGC
AA.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 11th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A32 that annotates to aldehyde dehydrogenase (NAD⁺) (*Nicotiana tabacum*) is 342 bp and showed the following sequence:

```
TTGGCGAGAAACATAACCATATTTACCTACA
ATCTGAAACAGGATTATGATTGATCTAAACA
TGGTTGGCTCTTGGGGGAGGATGACATGCCA
AAGCACAGGATGAGATTTTTGGTCCAGTGCA
GTCCATCTTGAAATACAAGTGAGCAATAAAG
CTTTCTTCTCTAAACCTGTTGGTATCCAATC
CCTTTTGTTAGAATTAACATTAACATTATGG
CTGATTGCAGGGACCTTGATGAGGTGGTAAG
AAGGGCAAATACTACGCGATACGGGCTTGCT
GCAGGGGTCTTACACAAAAACATAGATACTG
GAAACACATTGACACGTGGATTGGGGGTAA
A.
```

The NCBI BLAST search of this EST showed 18% similarity with both *Solanum lycopersicum* cDNA, clone: LEFL1042BA06, HTC in leaf and *Lycopersicon esculentum* clone 132363R, mRNA sequence.

The 12th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A35 that annotates to mevalonate diphosphate decarboxylase (*Hevea brasiliensis*) is 240 bp and showed the following sequence:

```
CCTTTCCATTTTTTTCATTTTGGTTGGAATG
GGGGGTGCGGGGTGAGGAAATTTTTTAAGTC
CTGATAAGGTCAAAGTTATTATGTTTGCTAC
TATATGGACCCCATGGGAATGGATTTCTACTA
TGGGGGTTTTTAAGTTCTCTTGGAAGTGGG
GTAATAAATCCCTTTTCCCCTGGATTCCATT
ATAAAAAACAAGTATTGGAGTTGGGGAAGA
TAATTCTCTAAATTTGTTGTCT.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 13th EST resulted from the amplification of Texas almond cultivar DNA using primer no. A36 that annotates to mevalonate kinase (*Hevea brasiliensis*)

is 694 bp and showed the following sequence:

```
TTTTTCCTTCCCATGGACCTCTACACCTATGT
CTCTCTTCGCTTTCCCACTCCTTCTGGGTA
CGCTCCTATCTCTCTCTGGCTATTTGGGGTT
TCTTCTTCTTCTTCTTCTTCTTCTTCTTTT
TCTTCCATCTATCCGATTTATTTATTTGGTT
TGGTCTGGTTTTTGGTGCCTTTGGGATTGGT
TTTGATTTGGGTTATGGTTGCGATGTCATTC
GGATACAATTTGAGTTCCTTTTTACCGTGTC
TCTCCTTGATCATTATTGTATTTTCTCTAT
TTGGGTTTCATGGGTTTTTCTTAAACTTTGTG
ATTTTACCTATGCAAAGTTTGTGTCTTTTT
GTGAGCTTAACATTTGCCTATTGGGTTGCTT
GAAATCATATGATTAAGAAACCCTTTTGA
TATGATGTATAAATCTATTTTCGTGGATTACT
CTGCTAGCCTAGTTGCAGGGTTTTAAGTGATA
TTAGAAATCTGATTGATTGCTTTCAGTAATG
TGTTTTCAAGAACGATAAATTTTTTGATCTG
GAAACTAATGGGATTCATATGCTCAAGTGAT
ATTGAATGAATTTCTCACTGCTGTTTCTGTC
TTTTTTTCATAGACAAATGATGATGCACTAAGA
CTCCAGCTCAAGGATGTTGGATTAGAGTTTT
CATGGCCAATTGGTAGAATAAAGAAAGCCCT
TTCCAGACAAAT.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 14th EST resulted from the amplification of Texas almond cultivar DNA using primer no. A37 that annotates to lipoxygenase (*Nicotiana attenuate*) is 198 bp and showed the following sequence:

```
GCATTGTGAATTGGTCCGAACCTATGTCAAT
TACTACTATCCTGATGCAAGTGCGGTTAATT
TTGATACTGAAGTGCAGGCCTGGTACAATGA
GTCAATCAATTTAGGCCATGCTGATCTTCGC
CATGCTAGCTGGTGGCTAAACTCTTACTC
CAGATGATCTCACATCCATTCTCACCACCAT
CATTTGGGTCAA.
```

The NCBI BLAST search of this EST showed 98% similarity with *Prunus persica* lipoxygenase 1 mRNA.

The 15th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A38 that annotates to lipoxygenase is 285 bp and showed the following sequence:

```
TGAACATGATGGCTCTAAACAAAAAAGGGA
ATCATGATCATTCTCCACCACAAGGCCTA
AGGAAAAGTGTCTTGTTTTACAGCTTGTTAG
TACTGAAACTGAAGCAGGTAAGCTATACAA
CTACTATTATCGTATTATTATTATTTATCA
ATATTAATTTTATTTTTTTTATTTTACAATAT
TTCTCTTTGGTGCCATATCTTTCTTCTTGA
CTTTTCTTCTTAATGTGCGCATTGAAACAAAA
GAGTCCACGAAGCCAAAGCTGAGCAATGAAG
CTTAAC.
```

The NCBI BLAST search of this EST showed 40% similarity with *Prunus persica* lipoxygenase 1 mRNA.

The 16th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A39 that annotates to acetyltransferase-like protein (*Arabidopsis thaliana*) is 276 bp and showed the following sequence:

```
TATCTTTGGTGAGGCTTCTAGTCGAGACATC
ATCTATAACTCCTCATTCCTCGAGAACCCTA
CAGCTCTCTGTTTTGGATCAGATGGTTCTTA
GTCACGTTTACTTCCCAACGCTTCTCTTCTA
TTCCGGAACAATAATATTACTGGTTCAGGAG
GTGGAGCTACTTCTACAGACATGGCGGCCAT
GAGGATGGAGAAAAATTATTGTGTCATCTAA
TTGGGTCATTAGCTAAAAATCTTTCACCTTCT
ACCCCTCGCAGAAAAATTAAGTGAAAA.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 17th EST resulted from the amplification of F₁ of cv *Texas* (almond) x cv *Earlygold* (peach) DNA using primer no. A41 that annotates to nicotinate phosphoribosyltransferase-like protein (*Medicago truncatula*) is 194 bp and showed the following sequence:

```
CTCTTTTTTTTATACTCAATTTCCACATTGGG
GGTTATGTTCCCTCAAGTGTTCGGGTTTG
AATTAATAATTATAATACTTTATCTGAGAGGG
TGGGGTCCCCAGCCCCTCCGCCTCCTAAAAG
GATTGGGGTTTCCAAATTAAATTTTTTTTGT
CTATGCCTTCATATGGGTATCCTGGGAAAGG
GAAAAAAA.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 18th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A42 that annotates to serine O-acetyltransferase 1 (*Glycine max*) is 159 bp and showed the following sequence:

```
TGGCCAGGACTTGTATTTTGGGGACATCAAA
ATTGGTGAAGGGGCAAAGATTGGGGCTTGTT
CTGTGGTTCTAAAGGAAGTGCCTCCAAGGAC
TACTGCAGTTGGGAACCCAGCTAGGCTGCTT
GGAGGGAAAGAACCACCCCTTTTGGGCC
ACAA.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 19th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A44 that annotates to alcohol acyl transferase (*Pyrus communis*) is 159 bp and showed the following sequence:

```
CCCGAGAAAATAGGACGCCAGGACGGCCTTC
GGTTTCTTTTCAGTCATCATATCTTATAAAA
ACAATCCTTCAATGAAAGGAAACGACGCCGT
TATGGTGATCAAGGAAGCATTGAGTAGAGCA
CTAGTGGATTACTACCCTTTGGCTGGGAGAC
TCAG.
```

The NCBI BLAST search of this EST showed 94% similarity with *Prunus armeniaca* alcohol acyl transferase (AAT) mRNA.

The 20th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A47 that annotates to 3 -ketoacyl-CoA thiolase B; acetyl-CoA C-acyltransferase (*Mangifera indica*) is 211 bp and showed the following sequence:

```
GGTAAAAACAGAAAATTTGGTCTCCTGGGTG
ACTGTCCTTTCATTTTTCTGTAATTAACTATT
TGTATGCATAGATTGTGGATCCGAAAACCTGG
AGAGGAGAGGCCTGTTACAATTTCTGTGGAT
GATGGGATCCGGCCAAATGCAAACATGAATG
ATTTGGCAAAGCTGAAGCCTGCGTTTTAAAGC
AGATGGGTCTACAACCTGCAAGAAAA.
```

The NCBI BLAST search of this EST showed no significant similarity.

The 21st EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A49 that annotates to N-myristoyl transferase (*Arabidopsis thaliana*) is 183 bp and showed the following sequence:

```
ACCAGTTACTGGCATAGGGTCTTTGACCCAA
AGAAGCTTATTGATGTTGGGTTTTCTAGGCT
TGGTGCCAGGATGACTATGAGCCGAACCATA
AAACTGTACAAGTTACCAGATTACCAGCTA
CTCCTGGATTGAGAAAATGGAACCTTCGTGA
TGTCCCTGCTGTAACCTCGGTTGCTTAGA.
```


The NCBI BLAST search of this EST showed 98% similarity with predicted: *Vitis vinifera* hypothetical protein LOC100256549 (LOC100256549), mRNA and 88% similarity with *Vitis vinifera* contig VV78X105607.9, whole genome shotgun sequence.

The 22nd EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. A50 that annotates to lipoamide dehydrogenase (*Pisum sativum*) is 237 bp and showed the following sequence:

```
CGGTAAGGGGGAACCTTTCAACCTCGGTATTC
AACACCAAGGGACTTCACTTGTTCCTCGGTC
TTTCCAACAAATGCAACTTCAGGGGGGTAT
AGACAACCCCAGGGGCCAAGTCATAGTCCAC
ATGCCAACCTTACCAGCAAGGGACTCCACG
CATGCAACCCCATCTCTTCTGCCTTGTGGG
CTAACATAGGTCCAGGAATAACGTCCCCGAT
TGGATAAACACCTGGGAAAA.
```

The NCBI BLAST search of this EST showed 93% similarity with the following three sequences; *Populus trichocarpa* precursor of dehydrogenase dihydro-lipoamide dehydrogenase 1 (LPD1), mRNA, *Populus trichocarpa* x *Populus deltoides* clone WS01314_P09 unknown mRNA and *Populus tremuloides* mitochondrial lipoamide dehydrogenase (LPD1) mRNA; nuclear gene for mitochondrial product.

The 23rd EST resulted from the amplification of F₁ of cv *Texas* (almond) x cv *Earlygold* (peach) DNA using primer no. 16 that annotates to pectinesterase 2 precursor is 343 bp and showed the following sequence:

```
GGGGCCAATTTAAAGGGCTGAAGGCTAGGGA
GTACGGAGCTGTCAAGGACTGCTTGGAGGAG
ATGGGTGATACCGTGGACAGGCTCAGCAAAT
CAGTCCAGGAGCTAAAGAACATGGGCAAATC
CAAGGGCCAGGATTTCTGTGTGGCACATGAGC
AATGTGGAGACTTGGGTTAGTGCTGCTTTGA
CTGATGACAATACTTGCCTTGATGGGTTCTC
TGGCAAGGCCTTGGATGGCAAATCAAGGCC
TCAATCAGAGCTCAGGTGCTTAATGTTGCAC
AGTGCCTAGCAATGCTTTGGCCTTGTGCAA
CAGGTTTGCCTCCAAGCACTGATGACAGCTT
AA.
```

The NCBI BLAST search of this EST showed 46% similarity with *Populus trichocarpa* predicted protein, mRNA and 17% similarity with *Populus trichocarpa* predicted protein, mRNA.

The 24th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. 19 that annotates to anthocyanidin synthase (*Prunus persica*) is 353 bp and showed the following sequence:

```
ATGGGGGGGGGACTACTTCTTCCACCTTTG
TGTACCCTGAGGACAAGCGTGACTTGTCCAT
TTGGCCTCAACACCTGCTGATTACATGGAAG
TACTCAAGGGAAAAAAAAAAATTCTTATTA
TGCGTGTGATTGGAAGATTTGTTGAAAAATC
TACCAAAAAAAAAACAACCTCTTTAACAACCGA
ATAATGGGGGAGGGGGGTTTTTTCATAAGAA
AAAAATGAGGGTTATCAACAATTTTGTTTTT
TTGACGCTCACCATTCTTGAGAAAAAAGGT
AACCTTTTTTGGGGGGGGCCCCGAGAAGTAG
GGAGTGGGGGGGGGTAAAAACAAGTGGGCGG
GGGGGAATTTT.
```

The NCBI BLAST search of this EST showed 49% similarity with the following three sequences; *Prunus persica* leucoanthocyanidin dioxygenase (LDOX) gene, complete cds, *Prunus persica*

leucoanthocyanidin dioxygenase (LDOX) gene, LDOX-1 allele, complete cds, *Prunus cerasifera* anthocyanidin synthase (ANS) gene, partial cds and 30% similarity with *Prunus persica* leucoanthocyanidin dioxygenase (LDOX) gene, LDOX-2 allele, complete cds.

The 25th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. 37 that annotates to catalase (*Prunus persica*) is 214 bp and showed the following sequence:

```
CGGCCTTGAAGATTTTCTTTCCCTGCAGCC
AGTTGGCCGTTTGGGTCTGAATAAAAAACATC
GATAACTTCTTTGCAGAGAATGAACAACTTG
CGTTTAAACCCTGCCCATGTTGTCCCTGGTGT
CTACTATTTCAGATGATAAGATGCTCCAAACT
CGAATCTTCGCCTATTCTGATACTCAGAGGC
ACCGTTTTGGCCCGAAAAAATAA .
```

The NCBI BLAST search of this EST showed 92% similarity with the following four sequences; *Prunus persica* mRNA for catalase 1, partial, *Prunus persica* mRNA for catalase (cat2 gene), *Prunus avium* catalase (cat2) mRNA, complete cds, *Prunus persica* mRNA for catalase (cat1 gene), 91% similarity with *Prunus persica* mRNA for catalase (cat1 gene), 88% similarity with *Zantedeschia aethiopica* catalase 1 (cat1) mRNA, complete cds and 80% similarity with *Zantedeschia aethiopica* catalase 1 (cat1) mRNA.

The 26th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. 71 that annotates to putative sodium-dicarboxylate cotransporter protein (*Arabidopsis thaliana*) is

445 bp and showed the following sequence:

```
GGGAAGGCAGTTCATATTTTGCATTGCG
CCTGTGACTGTCCCATAAGTAGCACCATCA
CTGAGTTTACCTCTTACAACCTCCACCACCAC
GCTGGTTGGGCTCTTCTAATTCAAATAACC
AAAATCATGCATGTAAATCCACTCCTCCTTA
TGGTTCTGGAGCCATTGGGGCAGAATTTTC
TTTCTTGGTTCCACAGGAACACCTTCAAATG
GATTGGATTTACCCTGCGCCACATTGAAATC
CAAAATATGAAAAACCGGGTTGCCATTAAA
AATTGCAGAATTGCTGTGGTCTCCCTTTTGA
TGCCACACTTGGAACATTCCTAATGGCTCAG
TTTCAACTAATATTTTTTTTATTTAACATTTT
CACAAGTCACTAATGAATTTTTGGCTATTGC
GGAACAATGTTTTGGGACTAACGGGACCAAT
TCAAAAAATAA .
```

The NCBI BLAST search of this EST showed 97% similarity with *Prunus persica* (peach) BAC clone 82I18, complete sequence.

The 27th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. 132 that annotates to cysteine protease (*Anthurium andraeanum*) is 661 bp and showed the following sequence:

```
AGGTTCTTCTGGGCTCAAGGGTAGGGAAAT
TCTATCATTCGTTTCATTTCATATCTACTT
GGTGTGTTGACACTGAAAACCAACATTATAGT
GTACTTCGCAAGTTGCAAATAACTTTAAC
CTAGTGTAGCAAGTTATGAAGCATATTAGAA
AAGGGAAAAAGTCGTCAAGTGTATACAGAA
TCATTTTCAATTTACCTCTCAAGGTAGATACAT
AGAATCAATAGAGCCGGCCGTTAAACTAATA
ACTAATGTCTCTCATATTTTCATTACACAGA
ATGCGGGAGATTTCTGGGGAGTATCTGCAA
GAAGAGAAAAATGGCCAAGCACAAGCTCCCA
TGGACTAAAGTAGAGCAAACAGAGAAGACAT
ACCACCCACTGCAGTGGGAAGATGAACCGGTT
```

TGCTGCAATGCGCTGAAAAAGGGAAAGGAAG
 TTATTGTTTCTGTGAAGTTTGAAGAAGGTTG
 TCACTATTGGTATTTAAATTTATGTAAAAACA
 GGGATTTGTTCCCTCTGGTTTGTTTTTTTGG
 TTTTTTAACAAATGTTCTTGGGGAGCATTGG
 TTCTTAAACATATGGGGGAACGAAAAACC
 TGAAAAAGAAAAATATTTTGAGAAAAGATG
 ATTTTTTCTCCTGCCGTTGGATGCAGCGGAG
 AAAAAAAAAA.

The NCBI BLAST search of this EST showed no significant similarity.

The 28th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. 144 that annotates to Loring-Oro-Red haven is 777 bp and showed the following sequence:

TCCCTTTTTCTATCTTTGTGGTTCATGTTAT
 CAGCCTGTGCGCTTTTCTCAAAAACATGGAT
 TGAAGTTTCTGGATCCTCTGCCAAGGATGTT
 GCCAAGCAGCTCAAGGTATTAATCTGAATA
 TATCAGTTATGTTTTCTCTAATATAACCAAG
 AAAGTGTATATTTTTGTGGGATCTCTTT
 CTGCAAATGAGGGGTTTGTGTGGGTAGAT
 ACCTACCCGTTATATGTATTTTCTAATATAT
 TTCTTATGATTTGTTTCACTTGATGCTTTTCG
 AGTTAATGACCAGATCATCTTTTTTTTATT
 TTCATACCGGAAGCGCTATTTTTATAATAAA
 GGGGAGTTGAACAATTCTTAAGTCAGAACT
 TTATGGATTATACTCAGAGATAAGCATCATG
 CTAACAAAATCCCTTTATAATTTGCAAGAAC
 AACAAATGGTGATGCCTGGTTCATCGTGAATC
 AACTTGCAAAAGGAGTTGAACCGCTACATT
 CCCACAGCTGCTGTTTTGGAGGCATGTGCA
 TCGGAGCACTGACAGTGTGGCCGATTTCTT
 GGGCGCAATTGGTTCAGGAACATGAATTCTG
 CTTGCAGTGACAATCATCTATCAGTACTTTT
 AGACATTGAGAAAGAAAGAGCTTGGAGCTC
 GATTTCTCTATGATTCCATGCAATACTGTTG
 CCAGAGATGGTGGGTCTCACCGACGATTTCT
 GGTGTGATCGAGCAATTTGTTGCCAGTAATG
 TTTGGTCTCCCTGACAATTTGTTGGTGTGCTG
 AG.

The NCBI BLAST search of this EST showed 27% similarity with both *Solanum lycopersicum* chromosome 2 clone C02SLe0011K05, complete sequence and *Solanum lycopersicum* cDNA, clone: FC03DH06, HTC in fruit.

The 29th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. 147 that annotates to glucose acyltransferase (*Lycopersicon pennellii*) is 632 bp and showed the following sequence:

AGAAAAACAGGAGCCGTCCTTTTCGTTTCGTC
 CCGATATTATACCTCATAAAATCTGCATTTT
 TAGATGAATTATTATATTTTCTAATTTTATG
 AAATTTTATAATCAGAAAATCTTTTTTAAAG
 AAAGGGAAGGCTCTGTTTTCCCTGTTTTTTT
 TGTTCTTTTTTGGGTTAGGCAGGGCGCCAAA
 GCACACAAGTAAACCTAATGAGCAACTAACC
 GAGCCCAAGAGCCTCAAAACCTATCAAAAGC
 CATACAAAGTTGATTAGCGTTAGCTTTCTAA
 GTATATTCTGTGATTTTTCACTAATACACA
 GAATATAATTGTAACCTCTGCACATTAAAGTT
 CCAACTGTTTAAACACATACTTTGTTGTAGGT
 CCCCTATCCTTTGACTATGCACATTCCATTG
 GCAACAAACAAAATTTAAATTTGAATCCATA
 TTCCTGGACAAAGGTAGGTTTTTCGTGTTTGT
 GTCTTTGTATGTGATCTTTAAAACCTATTCAG
 GTGCGTATGACATTGAGAAGGAAAATATCCC
 AAAACTTTCAGTTGCCACATAATATTTTTT
 TTAGACGCACCAGGGGCACAGGGATTCTCC
 TTATGCGAAAAAATTTGGGAAAGGATATAGC
 AATTCTCATGAC.

The NCBI BLAST search of this EST showed no significant similarity.

The 30th EST resulted from the amplification of Earlygold peach cultivar DNA using primer no. 160 that annotates to fantasia-Bolero-Red haven is 305 bp and showed the following sequence:

GTGCAAGCCCTACCTCTTTATTACGCTGTAA
 CTCGAGACGAATTGCTAGGGATGGCGGGAGA
 GGTATTTGGTAATGTTCAATCAGGTGATTG
 CGGGTTCGAGTAAATCACACCTACCCATTGT
 CTCAAGCAGCACAGGCACACGAAGACCTTGA
 GAATAGGAAAACATCTGGATCTGTTGTGCTT
 ATCCCTAAGGCAAATATGAGTTTGGTCTGT
 TCATTTTAAAGGACTCGTGGGGGTGTGTGA
 AAATGAATAAGGAACGTTTGCCTTCTGTTC
 CGGATAGCTGCTGTGCCAATCAAAA.

The NCBI BLAST search of this EST showed no significant similarity.

DISCUSSION

EST localization derived from candidate genes for a specific function in genetic maps. Therefore, peach and almond functional genomics has been very important due to the effort to improve peach fruit properties such as flesh softening, ethylene metabolism, aroma productions, nutraceutical, etc. However, the genomic databases of peach and almond and their progeny can play a significant role in the gene discovery and the genetic understanding of related species (Lazzari *et al.*, 2005).

In the current study, the thirty ESTs were sequenced for the first time and so far, they were not submitted to the databases. Consequently, 17 ESTs (13 belong to Earlygold peach cv., three belong to Texas almond cv. and one belongs to their F₁ hybrid) -and resulted from the amplification using the following 17 primers; A7, A12, A16, A20, A21, A22, A25, A27, A35, A36, A39, A41, A42, A47, 132, 147, 160- out of the thirty ESTs showed no significant

similarity when they were subjected to the BLAST search in the ncbi web site for sequence alignment. On the other hand, thirteen sequences (that resulted from the amplification using the following 13 primers; A23, A24, A32, A37, A38, A44, A49, A50, 16, 19, 37, 71, 144) had different levels of similarities ranges from 19 to 99%. Yhis results are consistent with (Lazzari *et al.*, 2005). The distribution of these 13 ESTs is as follows; 10 belong to Earlygold peach cv., two belong to Texas almond cultivar and one belongs to their F₁ hybrid.

The sequenced EST of the current study will be submitted to databases to be used as a resource for ESTree (Lazzari *et al.*, 2004, 2005, 2008) and genome database for *Rosaceae* (GDR) (Jung *et al.*, 2004). The ESTree database pipeline was used in EST analyses for related projects, with different input datasets; data flow was maintained through the entire process, but allowing the preparation of dataset-specific outputs. The contig assembly process was kept apart from the putative SNP detection procedure, allowing the two processes to be carried out independently. In some cases, different features were added and easily integrated in the procedure; i.e. BLAST analysis versus species specific genomic sequences (Lazzari *et al.*, 2004). On the other hand, Lazzari *et al.* (2008) introduced version VI of ESTree database. This ESTree database offers a broad overview on peach gene expression. They reported that EST provides systematic sampling of the transcribed

portion of the genome, provides “sequence tags” allowing unique identification of genes, provides experimental evidence for the positions of exons, provides regions coding for potentially new proteins and provides clones for DNA microarrays. On the other hand EST has some limitations; some cDNA are over-represented and rediscovered many times before a weakly expressed gene can be identified, partial representation due to tissue-specific and developmental regulation of gene expression and non-overlapping reads from the 5' end are scored as independent genes (Lazzari *et al.*, 2008).

SUMMARY

A total of 30 successful ESTs (expressed sequence tags) were amplified and sequenced to be a resource for *Rosaceae* functional genomics data base. 23 EST were isolated from the amplification of Earlygold peach (*Prunus persica*) cultivar DNA, 5 ESTs were isolated from the amplification of Texas almond cultivar and two ESTs were isolated from the amplification of F₁ DNA of their hybrid. All the sequences were tested for similarity using BLAST in the NCBI (National Center for Biotechnology Information) database. Because these sequence data are new, only 13 sequences found similarity (10 belong to Earlygold peach cv., two belong to Texas almond cultivar and one belongs to their F₁ hybrid), whereas the other 17 (13 belong to Earlygold peach cv., three belong to Texas almond cultivar and one belongs to

their F₁ hybrid) shown no significant similarity. The resulting database will be used as a resource of data and links related to peach and almond EST sequence databases.

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Table (1): The EST primers used in EST amplification in this study.

Primer	Sequence	Primer	Sequence
A7for	GCCATGGGTGCTCTAGATCATC	A39for	ACAAAGTAAGATGGCCCCAGAC
A7rev	TGGCCAGAACTCCACCTTCTTG	A39rev	ACTCAATCTTCCTGCTAGAGGG
A12for	TCTTGCCAACATCTTTCTGCCC	A41for	TCGTAGCAGTGATAGCCAGAGC
A12rev	TTCAGGGAAGGCTATCAGAACC	A41rev	TAATGCCGGCTGCTTTGTACCC
A16for	GCGATAAAGCAAGTCGATGTGG	A42for	GGCACCCAAAGATTGGTGATGG
A16rev	GTAGCCAGCAAAGAAGTTGGAG	A42rev	TCTTTCCTCCAAGCAGCCTAG
A20for	TGTGCGTAACAGGAGCATCTGG	A44for	CGAGCCGGAACCTTATAACACCG
A20rev	TCTGTCTGTGGGTCAGTGGATG	A44rev	GAGTCTACCAGCCAAAGGGTAG
A21for	TTGATGCTCTCAAAGGCTGCTC	A47for	GCAACAGCATCAGGCCAAATTCC
A21rev	ATCAGTCCAGTGTCTGTCATCC	A47rev	TTCCTGCAGTTGTAGACCCATC
A22for	AGTGATCCCAAGGATTGGCAGG	A49for	CAGCTGGAGTAGTTCTTCCAAC
A22rev	GTCCCACACATGAACCTCAACG	A49rev	CTAAGCAACCGAGTTACAGCAG
A23for	TTTTTTCCTCAGCAACCCGGG	A50for	GTCCCAGGTGTTTATGCAATCG
A23rev	GAGGGCTGCACTGAAACATACG	A50rev	TGACGAGTCCTCAGCATCATC
A24for	TTCCCCTGGCTATTGAGACTGC	16-F	AGCCTAACCCAGGGCTCAAAGG
A24rev	AGTCGACAGCCAAATCAGCACC	16-R	AGCTGTCATCAGTGCTTGGAGG
A25for	TGACATCAACTCGGCTGTTGGC	19-F	GGCCTCTGGCAAAATTCAAGGC
A25rev	CCTCAACAATGACACCAGAGCC	19-R	TGGAACACACTTGGCAGTGACC
A27for	CGGCAAACAGAGATGAAGAGCG	37-F	TGGATCCTGATCACGAGGACAG
A27rev	GCCACAACATCACAGTGGTAGG	37-R	ATAGTTCGGGCCAAAACGGTGC
A32for	GAGGCTTGGCACAAAGGGTTTC	71-F	TTTGCCATTGCCGACGGAGTTC
A32rev	ACCCGCAATGCACGTGTCAATG	71-R	TTGAACTGGTCCCGTTAGTCCC
A35for	GCGAATGAAGATCACAGCCAGC	132-F	TTGCTGCACCGGAACCTGACTAC
A35rev	CGCATTCCTGTAGTGCTACTCG	132-R	TCTTGTCCGTTGCATCAACGCAG
A36for	TTCCTCCATTGACCTCTACACC	144-F	AAGCTTAGCTGATATGGCAGCC
A36rev	ACATGAGGTTGGCACTGATGAG	144-R	CAGCACACCAAATGTGAGGG
A37for	CCCTTATGCAACAGATGGACTC	147-F	GTATGATCCTCTGGTGCTTTGG
A37rev	TGACCCAAATGATGGTGGTGAG	147-R	TGCCGAGCACATATCCTTTGAG
A38for	CTACTGCCGTTTATCAGACGAC	160-F	TCCATTGTCAGCCATTGCAGTG
A38rev	AGCTTCATTGCTCAGCTTTGGC	160-R	TGATTTGGCACAGCAGCTATCC

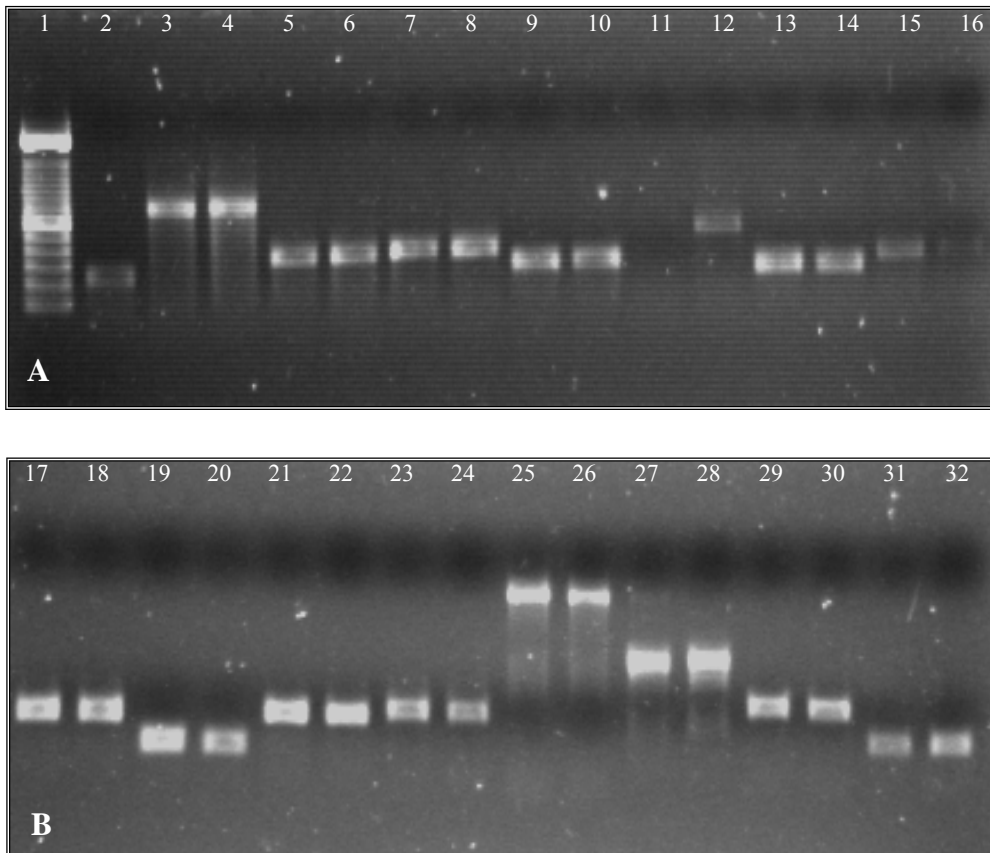


Fig. (1): A and B are examples of amplified ESTs in the ESTree project, some of them were sequenced and presented in the current investigation.

- | | | |
|-----------------------|-----------------------|-----------------------|
| 1. DNA ladder | 2. A19Texas almond | 3. 144Earlgold peach |
| 4. 144Texas almond | 5. 160Earlgold peach | 6. 160Texas almond |
| 7. A38Earlgold peach | 8. A38Texas almond | 9. A47Earlgold peach |
| 10. A47Texas almond | 11. A48Earlgold peach | 12. A48Texas almond |
| 13. A49Earlgold peach | 14. A49Texas almond | 15. A50Earlgold peach |
| 16. A50Texas almond | 17. A24Earlgold peach | 18. A24Texas almond |
| 19. A42Earlgold peach | 20. A42Texas almond | 21. A25Earlgold peach |
| 22. A25Texas almond | 23. A27Earlgold peach | 24. A27Texas almond |
| 25. A29Earlgold peach | 26. A29Texas almond | 27. A23Earlgold peach |
| 28. A23Texas almond | 29. A32Earlgold peach | 30. A32Texas almond |
| 31. A44Earlgold peach | 32. A44Texas almond | |