Full Length Research Paper

Amplification and sequencing of *Rosaceae* expressed sequence tags (ESTs) as a resource for functional genomics databases

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A total of 30 successful ESTs (expressed sequenced tags) were amplified and sequenced to be intended as 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 nbci 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) found no significant similarity. The resulting database can be used as a resource of data and links related to peach and almond EST sequences.

Key words: Peach, almond, expressed sequence tags, functional genomics.

INTRODUCTION

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 years) 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 and 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 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 (EST) is 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 that is, 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 of high-guality importance fruit and the

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intrinsic difficulties of breeding in a perennial species require the development and application of structural and functional genomic databases for the sustained improvement of rosaceaous 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 an Expressed Sequence Tags (ESTs) database that was developed by the Italian ESTree Interuniversitary 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 data set 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 F_1 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/ $\mu l).$

PCR cycle profile

Primary denature at 95 $^{\circ}$ C for 3 min for one cycle and 35 cycles with the following profile: 95 $^{\circ}$ C for 45 s, 57 $^{\circ}$ C for 1 min and 68 $^{\circ}$ C for 1 min, and a final extension at 68 $^{\circ}$ C for 5 min. Two µl of the PCR product were then visualized on ethidium bromide stained 1.5% agarose gel and photographed (Figure 1).

DNA sequencing

The PCR product was purified, quantified (and adjusted as 10 ng/ μ l) 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 (Table 1). 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:

TGATGCTCGGGGTGGGAGTTCAGGCTCAGGAACGC AAGCAATTCCAGGTATTTTTTTTTCCTTCACAAAATCTC CATATCCAAAAAGGGCTTATAAAAAGTAAATCAAGAA AATCCAAAGTGATTTTTAAATTTTTTTTTTTTTTGGGGG CGGAAAAAAGAATTTTTTTTTTTTGTTGTTGGA GGGTTTTTTTTTTTTTTTTTTGGAATTTCCAAAAGGGG AGGCAAGGGATCCCAACACCCCAAAAGTTGTGGGCT TTTTTTTTTTTTTAAAAAAAAACTTTTTTTTTATTCTTTT TAGCGGGTAGCTCCCCCAAAACCCCCAAAAAAAAAA CAACGGGGGGGGGGGCCCGCCTGTACAAAACCAACTAA ACAACAATACTTCTTTGCCGACATTTTGGTTTTTTT CCCACGGGGGGAAACCCCGGAGAAAAAGGCCCGCTAA GAACGAGAGAGGGGGGAAGTAAAACCTGGTGAAAGG GGAAAGAAGGGGACGCCAGTGAGGGCGGAGAAAAG GCCCCCCATACACCTTCCCCGGCAATTAGGACCCCC AGGGGGGAGAAAAGGGGGGGGTTTTCGCGCCGCAAAA AA.

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:

TCCATTACCGTTGGGAGGACTCCTACAATGGCTCTC GATCATGATGAGTTCAAAACTGATATCTGAGGCATCC TTAATAAACAATTGGATCTTTCTTCATTGTCTGAAATC ATTAATCAAAACGGATTAAAAGAATTGATAATTTTAT CACAACCTGAAGATGTTAATTATATATGGGGGGACCAC TCACATTGGATATGTCTACCCCTTGATCAAGGGGGCT GATAGCCTTCCCTGAAAA.

Primer	Sequence	Primer	Sequence
A7-F	GCCATGGGTGCTCTAGATCATC	A39-F	ACAAAGTAAGATGGCCCCAGAC
A7-R	TGGCCAGAACTCCACCTTCTTG	A39-R	ACTCAATCTTCCTGCTAGAGGG
A12-F	TCTTGCCAACATCTTTCTGCCC	A41-F	TCGTAGCAGTGATAGCCAGAGC
A12-R	TTCAGGGAAGGCTATCAGAACC	A41-R	TAATGCCGGCTGCTTTGTACCC
A16-F	GCGATAAAGCAAGTCGATGTGG	A42-F	GGCACCCAAAGATTGGTGATGG
A16-R	GTAGCCAGCAAAGAAGTTGGAG	A42-R	TCTTTCCCTCCAAGCAGCCTAG
A20-F	TGTGCGTAACAGGAGCATCTGG	A44-F	CGAGCCGGAACTTATAACACCG
A20-R	TCTGTCTGTGGGTCAGTGGATG	A44-R	GAGTCTACCAGCCAAAGGGTAG
A21-F	TTGATGCTCTCAAAGGCTGCTC	A47-F	GCAACAGCATCAGGCAAATTCC
A21-R	ATCAGTCCAGTGTCGTTCATCC	A47-R	TTCCTGCAGTTGTAGACCCATC
A22-F	AGTGATCCCAAGGATTGGCAGG	A49-F	CAGCTGGAGTAGTTCTTCCAAC
A22-R	GTCCCACACATGAACCTCAACG	A49-R	CTAAGCAACCGAGTTACAGCAG
A23-F	TTTTTTCCCTCAGCAACCCGGG	A50-F	GTCCCAGGTGTTTATGCAATCG
A23-R	GAGGGCTGCACTGAAACATACG	A50-R	TGACGAGTCCTTCAGCATCATC
A24-F	TTCCCCTGGCTATTGAGACTGC	16-F	AGCCTAACCAGGGCTCAAAAGG
A24-R	AGTCGACAGCCAAATCAGCACC	16-R	AGCTGTCATCAGTGCTTGGAGG
A25-F	TGACATCAACTCGGCTGTTGGC	19-F	GGCCTCTGGCAAAATTCAAGGC
A25-R	CCTCAACAATGACACCAGAGCC	19-R	TGGAACACACTTGGCAGTGACC
A27-F	CGGCAAACAGAGATGAAGAGCG	37-F	TGGATCCTGATCACGAGGACAG
A27-R	GCCACAACATCACAGTGGTAGG	37-R	ATAGTTCGGGCCAAAACGGTGC
A32-F	GAGGCTTGGCACAAAGGGTTTC	71-F	TTTGCCATTGCCGACGGAGTTC
A32-R	ACCCGCAATGCACGTGTCAATG	71-R	TTGAACTGGTCCCGTTAGTCCC
A35-F	GCGAATGAAGATCACAGCCAGC	132-F	TTGCTGCACCGGAACTGACTAC
A35-R	CGCATTCCTGTAGTGCTACTCG	132-R	TCTTGTCCGTTGCATCAACGCAG
A36-F	TTCCTCCATTGACCTCTACACC	144-F	AAGCTTAGCTGATATGGCAGCC
A36-R	ACATGAGGTTGGCACTGATGAG	144-R	CAGCACACCAAAATTGTCAGGG
A37-F	CCCTTATGCAACAGATGGACTC	147-F	GTATGATCCTCTGGTGCTTTGG
A37-R	TGACCCAAATGATGGTGGTGAG	147-R	TGCCGAGCACATATCCTTTGAG
A38-F	CTACTGCCGTTTATCAGACGAC	160-F	TCCATTGTCAGCCATTGCAGTG
A38-R	AGCTTCATTGCTCAGCTTTGGC	160-R	TGATTTGGCACAGCAGCTATCC

Table 1. The EST primers used in EST amplification in this study. F = Forward and R = Reverse.

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:

CGTCCGCCTCAGTTGGCTGACAAGGTACGATCATCG CTGCTATCAAGGAAGCTGGCAATGTTAAGGTAAAAGT TTCGATCTTTTATCTTGGTTTTCTCTGTTTTTGCTTCTT GTGTTCGTGTTTTCTGTGTTGCGATCGTGAATGTGTC GTTGGGTTTCAGAGGTTTTTCCCATCTGAGTTTGGAA ACGACGTGGATCGAGTTCATGCTGTTGAGCCAGCAA AAACTGCATTTGCAACCAAGGCCAAAATTCGCAGAAC GATTGAGGCTGAGGGGGATCCCTCACACCTATGTGGC CTCCAACTTCTTTGCTGGCGACAATAT.

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:

Α	-	nona	peach	puou	l peach	puou	d peach	nond	d peach	mond	d peach	nond	d peach	mond	d peach	mond
	JNA ladder	A 19 1 exas all	[44Earlgold	[44Texas alr	l60Earlygold	l60Texas alr	A38Earlygol	A38Texas alı	A47Earlygol	A47Texas al	A48Earlygol	A48Texas alı	A49Earlygol	A49Texas al	A50Earlygol	A50Texas al



A25Earlygold peach A27Earlygold peach A29Earlygold peach A23Earlygold peach A32Earlygold peach A44Earlygold peach A24Earlgold peach A42Earlgold peach A29Texas almond A32Texas almond A44Texas almond В A27Texas almond A23Texas almond A24Texas almond A42Texas almond A25Texas almond



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

AACTAATTACTTTTGTAATTATGCATATGCCACAGATG ACCCGAAGAAAACAGAACACTTATTGGCACTTGAGG GGGCAAAAGAAAGGCTCCATTTGTTCAAAGCAGATTT GTTAGAAGAAGGATCTTTTGATGCTGTTGTGATGGAT GTGAGGGTGTTTTCATACAAGCTTCC

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:

CCCCCCTTACAAGAGGGGGGGGGGGGGGGGGCGCCCCC GGTTTTTCATATGGAGGGAAGGAGTCATCGACTCCC CTTAATTTTTGTTGCGGGGGGGGGGATGATGGGCCTTTG GAACCATAAGATCACCCCTTCCAAAAAATTGGGGTCT GAATTTGGGTGGGGGAAAAAAGGGGGACAACATACAGT GAAAAATTTTGATTTGTGGCAACAAAGTATTTGGTTT GTGCCATTTCTCTTTTCAGAAAGAAACTCGCACTTTT TCGTTTCTACATTTAAGGAGAGAAAAAAATTGTCTTT TTTGCCCGTGTCAGAAATGGGATTTGAACCTCTCTT TTTTCTTATCCCAACCACCATAATGTTTTACAATGTAA TAAGTCCCTTACATCCCTTCCAGAAAAAAATTACGGG ATTAATATTTGTTGAAGAACACAGTCTTTTAGTCAAGA AAGCCAATAACGCACAGGGGTTTTATTTCTTTAAAGT AATAATTAATATTTTTTTTTTTCCATTAGGATTTTAAGAT ATAAATTAAGCTCCGCCACTGTTATCAGCCGACTTTC TCATGTTTTTGGTAGCCCAATTGGGTTTTGGACCTAA AAAAAAGATAAAATAAACCACCTTTCTTCCCGAAAAA AACCTCCACTATTAGGCAAATATTAAATTAAACAAGG TGTAATACCA

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:

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:

TTTTTTTTTTTTTTTCTCCCCCGACCGCCCCGGGGTG GCGTTCCTTCTCGCCACACCGCCACAATAAAAACAAT ATTTCTTGGGCGAAGCTATTTTCCAACCCTTATGTTC CCGAACTTGACCCGTAAAAGGGGGGTCAGTGAAAGAT AAAAGCAGTACCAAGGTAGCATTTTGATCTTCTTTAC AATTTATTTTAGTAAAAATACCTTCTTTTTTTTCCTTA TTTTGGGAGTAGGCCTTTCCTTTTTTTTAGTAAGCCT TACCAATCCGTTACAATCTCTTCCCCTCAACTCACCC CGACCTATTTAATTAAATCCAATCCCTTCGACCCTTT GAAGAGAACACCATTTCAGTCCATTGACCTGGAGCT GTGTTGAGATGAGGTCGAGCTATATCTTGTGTTGTTT TGCTTTCCATCATAACGTAGGGGATCAGCAACAAACA CTTCAGATTTGACGTTGTGAAGCTTATTGTTGTGGAC ACGAAACCCAACTCCTAAACTTAAGATATGCACATAG TTAAGCCAAAAAAAATATATATGCACATAGTTATGGAA AAATATATATATGCACATAGATTCAGTTGCAAAACCA CTTTCCAGGTGTCTAAGGCCTGGAATTTACCTCGGC ACCTTGTACTCAGGGTAAAGTTCAGCTACCTTGGCCA CGAAGTCCCCATAATGTGATATAGCTTCAACGCACAG GTGTCTACCAGTGGCCGATTTGTTCTCATACACTAAA ATGTGTGCAAGAGCTACATCTTTAAAATCCACCGGCC CTCAT

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:

TTTTGAACTGGTCCCGTTAGTCCCAAATACATATGCT CCTGCAATAGCCAAAAATTCATTAGTGACTTGTGAAA ATTGGTCAAAAACAAAATACTTAGCTGATAACTGAGA CACTAGGAATGTTACCAAGTGTAGGCATCAAAAGGG AGAGCACAGCAATTCCAGCAATCTTTAATGGCAACCC GGTTTTGATCATATCTTGGATTTCAATGTGGCCAGTG GTAAATCCAACTACATTTGAAGGTGTTCCTGTTGGAA GCAAGAAAGAAAATTGTGCCCCCAATGGCTCCAGGAA CCATAAGGAGGAGTGGATTTACATGCATGATTTTGGC TATTTGAATTAGAAGAGGCACAACCAGCGTGGTGGT GGAGTTGTTTGATGTAAACTCAGTGATGGTGCTACTT ATGAGACAGACGGCAGGCGCAATGGCAAAATATGGA ACTGCCCTCCCCA

The NCBI BLAST search of this EST showed 98% similarity with *Prunus persica* (peach) BAC clone 82118, 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 DDTFR10 (*Lycopersicon esculentum*) is 272 bp and showed the following sequence:

TGGGAAGGATGCTCATTCGTTTCATGCAGCTCTTTCA

AAGCCTCCATCATCAGAATTTGTGAACGTGTCTCGGT GGTACAACCATATCACTGCACTTCTTAGGATTTCGTA AGTTCTTATAACTTGTGATGGGTTTCTTTATCTTTAT TATTAGATTATGATGGATTTCTTCAAAGCTCATATATT TGGGTTAAATCTTTGACTTATTTGATTATCTCTGGTGG TGTAGGGGTGTTTCTGGACAAGGCTCTGGTGTCTTT GTTGAAGAAAA

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:

TTTGGATAAAACGCCCGGGATTTGCGTAGTGATTTTG ATGCAGCCGTTCCGTCGGCGAAATCAGGAAGAATCG GAGTGGGAATGGTTGAGGGGGGGGTGGTGCTGCCGTTG TATTGAAGAATGGCAGAGGTGGTGCTGTTGTTGAAT GCAACATCCCCATCAACAAAAGGATGGGAAGCTACG TGATAGTGGCTGGGAGACTGGTTTGCAACTACCAAA ATGTCCATGGTTTGGCCTGGAGTTATCATGAGGTAG GAGGTGGTTATAGGTTTTATGTATGCACCATCTTGAG CTACCACTGTGATGTTGTGGCAA

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:

TTGGCGAGAAACATAACCATATTTACCTACAATCTGA AACAGGATTATGATTGATCTAAACATGGTTGGCTCTT GGGGGAGGATGACATGCCAAAGCACAGGATGAGATT TTTGGTCCAGTGCAGTCCATCTTGAAATACAAGTGAG CAATAAAGCTTTCTTCTCTAAACCTGTTGGTATCCAAT CCCTTTTGTTAGAATTAACATTAACATTATGGCTGATT GCAGGGACCTTGATGAGGGTGGTAAGAAGGGCAAATA CTACGCGATACGGGCTTGCTGCAGGGGGTCTTCACAC AAAACATAGATACTGGAAACACATTGACACGTGGATT GGGGGGTAAA

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 disphosphate decarboxylase (*Hevea brasiliensis*) is 240 bp and showed the following sequence:

CCTTTCCATTTTTTCATTTTGGTTGGAATGGGGGGGT GCGGGGTCAGGGAATTTTTTAAGTCCTGATAAGGTC AAAGTTATTATGTTTGCTACTATATGGACCCCATGGG AATGGATTTCACTATGGGGGGTTTTTAAGTTCCTCTTG GAAGTGGGGTAATAAATCCCTTTTCCCCTGGATTCCA TTATAAAAATACAAGTATTGGAGTTGGGGAAGATAAT TCTCTAAATTTGTTGTTCT

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:

TTTTCCTTCCCATGGACCTCTACACCTATGTCTCTCT CGCTTTCCCCACTCCTTCTGGGTACGCTCCTATCTCT CTCTGGCTATTTGGGGTTTCTTCTTCTTCTTCTTCTTCTTC TTCTTCTTTTTCTTCCATCTATCCGATTTATTTATTTG GTTTGGTCTGGTTTTTGGTGCCTTTGGGATTGGTTTT GATTTGGGTTATGGTTGCGATGTCATTCGGATACAAT TTTGAGTTCTTTTTACCGTGTCTCTCCTTGTATCATTA TTGTATTTCTCTATTTGGGTTCATGGGTTTTTCTTAA ACTTTGTGATTTTACCTATGCAAAAGTTTGTGTCTTTT TGTGAGCTTAACATTTGCCTATTGGGTTGCTTGAAAT CATATGATTAAAAGAAACCCTTTTGATATGATGTATAA ATCTATTTCGTGGATTACTCTGCTAGCCTAGTTGCAG GGTTTAAGTGATATTAGAAATCTGATTGATTGCTTTCA GTAATGTGTTTTCAAGAACGATAAATTTTTTGATCTGG AAACTAATGGGATTCATATGCTCAAGTGATATTGAAT GAATTTCTCACTGCTGTTTCCTGCTTTTTTCATAGACA ATGATGATGCACTAAGACTCCAGCTCAAGGATGTTG GATTAGAGTTTTCATGGCCAATTGGTAGAATAAAGAA AGCCCTTTCCAGACAAAT

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:

GCATTGTGAATTGGTCCGAACCTATGTCAATTACTAC TATCCTGATGCAAGTGCGGTTAATTTTGATACTGAAC TGCAGGCCTGGTACAATGAGTCAATCAATTTAGGCC ATGCTGATCTTCGCCATGCTAGCTGGTGGCCTAAAC TCTCTACTCCAGATGATCTCACATCCATTCTCACCAC CATCATTTGGGTCAA

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:

CTTTGGTGCCATATCTTTCTTCTTTGACTTTTCTTCTT AATGTCGCATTGAAACAAAAGAGTCCACGAAGCCAA AGCTGAGCAATGAAGCTTAAC

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 acetyltranferase-like protein (*Arabidopsis thaliana*) is 276 bp and showed the following sequence:

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

The 17^{th} 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:

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:

TGGCCAGGACTTGTATTTTGGGGACATCAAAATTGGT GAAGGGGCAAAGATTGGGGCTTGTTCTGTGGTTCTA AAGGAAGTGCCTCCAAGGACTACTGCAGTTGGGAAC CCAGCTAGGCTGCTTGGAGGGAAAGAACCACCCCC CTTTTTGGGCCACAA

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:

CCCGAGAAAATAGGACGCCAGGACGGCCTTCGGTTT CTTTTCAGTCATCATATCTTATAAAAACAATCCTTCAA

TGAAAGGAAACGACGCCGTTATGGTGATCAAGGAAG CATTGAGTAGAGCACTAGTGGATTACTACCCTTTGGC TGGGAGACTCAG

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:

GGTAAAAACAGAAAATTTGGTCTCCTGGGTGACTGTC TTTCATTTTCTGTAATTAACTATTTGTATGCATAGATT GTGGATCCGAAAACTGGAGAGAGAGAGGCCTGTTACA ATTTCTGTGGATGATGGGATCCGGCCAAATGCAAAC ATGAATGATTTGGCAAAGCTGAAGCCTGCGTTTAAAG CAGATGGGTCTACAACTGCAAGAAAA

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:

ACCAGTTACTGGCATAGGGTCTTTGACCCAAAGAAG CTTATTGATGTTGGGTTTTCTAGGCTTGGTGCCAGGA TGACTATGAGCCGAACCATAAAACTGTACAAGTTACC AGATTCACCAGCTACTCCTGGATTCAGGAAAATGGAA CTTCGTGATGTCCCTGCTGTAACTCGGTTGCTTAGA

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:

CGGTAAGGGGGAACTTTCAACCTCGGTATTCAACAC CAAGGGACTTCACTTGTTCCTCGGTCTTTCCAACAAA TGCAACTTCAGGGGGGGTATAGACAACCCCAGGGG CCAAGTCATAGTCCACATGCCCAACCTTACCAGCAA GGGACTCCACGCATGCAACCCCATCCTCTTCTGCCT TGTGGGCTAACATAGGTCCAGGAATAACGTCCCCGA TTGGATAAACACCTGGGAAAA

The NCBI BLAST search of this EST showed 93% similarity with the following three sequences; *Populus trichocarpa* precursor of dehydrogenase dihydrolipoamide 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 23^{rd} 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:

GGGGCCAATTTAAAGGGCTGAAGGCTAGGGAGTAC GGAGCTGTCAAGGACTGCTTGGAGGAGATGGGTGAT ACCGTGGACAGGCTCAGCAAATCAGTCCAGGAGCTA AAGAACATGGGCAAATCCAAGGGCCAGGATTTCGTG TGGCACATGAGCAATGTGGAGACTTGGGTTAGTGCT GCTTTGACTGATGACAATACTTGCCTTGATGGGTTCT CTGGCAAGGCCTTGGATGGCAAAATCAAGGCCTCAA TCAGAGCTCAGGTGCTTAATGTTGCACAGTGCACTA GCAATGCTTTGGCCTTGTGCAACAGGTTTGCCTCCA AGCACTGATGACAGCTTAA

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:

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:

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:

The NCBI BLAST search of this EST showed 97% similarity with *Prunus persica* (peach) BAC clone 82118, 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:

AGGTTCTTCTGGGCCTCAAGGGTAGGGAAATTCTAT CATTCGTTTCATTTCAATATCTACTTGGTGTTTGACAC TGAAAACCAACATTATAGTGTACTTCGCAAGTTGCAA ACTAATACTTAACCTAGTGTAGCAAGTTATGAAGCAT ATTAGAAAAGGGAAAAAAGTCGTCAAGTGTATACAGA ATCATTTCATTTACCTCTCAAGGTAGATACATAGAATC AATAGAGCCGGCCGTTAAACTAATAACTAATGTCTCT CATATTTTCATTACACAGAATGCGGGAGATTTCTGGG GAGTATCTGCAAAGAAGAGAAAAATGGCCAAGCACA AGCTCCCATGGACTAAAGTAGAGCAAACAGAGAAGA CATACCACCCACTGCAGTGGAAGATGAACCGGTTTG CTGCAATGCGCTGAAAAAGGGAAAGGAAGTTATTGT TTCTGTGAAGTTTGAAGAAGGTTGTCACTATTGGTAT TTAAATTTATGTAAAACAGGGATTTGTTCCTCCTGGTT TGTTTTTTGGTTTTTTAACAAATGTTCTTGGGGAGCA TTGGTTTCTTAAACATATGGGGGGGAACGAAAAACCTG TAAAAGAAAAAATATTTTGAGAAAAGATGATTTTTTCT CCTGCCGTTGGATGCAGCGGAGAAAAAAAAAA

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:

TCCCTTTTTCTATCTTTGTGGTTCATGTTATCAGCCTG TGCGCTTTTCTCAAAAACATGGATTGAAGTTTCTGGA TCCTCTGCCAAGGATGTTGCCAAGCAGCTCAAGGTA TTAAATCTGAATATATCAGTTATGTTTTCTCTAATATA CCAAAGAAAGTGTTTATATTTTTGTTGGGATCTCTTTC TTGCAAATGAGGGGGTTTGTGTGGGGTAGATACCTAC CCGTTATATGTATTTTCTAATATATTTCTTATGATTTGT TTCACTTGATGCTTTCGAGTTTAATGACCAGATCATC CTTTTTTATTTCATACCGGAAGCGCTATTTTTATAA TAAAGGGGAGTTTGAACAATTCTTAAGTCAGAACTTT ATGGATTATACTCAGAGATAAGCATCATGCTAACAAA ATCCCTTTATAATTTGCAAGAACAACAAATGGTGATG CCTGGTCATCGTGAATCAAACTTGCAAAAGGAGTTGA ACCGCTACATTCCCACAGCTGCTGCTTTTGGAGGCA TGTGCATCGGAGCACTGACAGTGTTGGCCGATTTCT TGGGCGCAATTGGTTCAGGAACATGAATTCTGCTTG CAGTGACAATCATCTATCAGTACTTTTAGACATTCGA GAAAGAAAGAGCTTGGAGCTCGATTTCTCTATGATTC CATGCAATACTGTTGCCAGAGATGGTGGGTCTCACC GACGATTTCTGGTGTGATCGAGCAATTTGTTGCCAGT AATGTTTGGTCTCCCTGACAATTTTGGTGGTGCTGAG

The NCBI BLAST search of this EST showed 27% similarity with both *Solanum lycopersicum* chromosome 2 clones 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:

AGAAAAACAGGAGCCGTCCCTTTCGTTCGTCCCGAT ATTATACCTCATAAAATCTGCATTTTTAGATGAATTAT TATATTTTCTAATTTTATGAAATTTTATAATCAGAAAAT CTTTTTAAAAGAAAGGGAAGGCTCTGTTTTCCCTGTT TTTTTTGTTCTTTTTGGGTTAGGCAGGGCGCCAAAG CACACAAGTAAACCTAATGAGCAACTAACCGAGCCC AAGAGCCTCAAAACCTATCAAAAGCCATACAAAGTTG ATTAGCGTTAGCTTTCTAAGTATATTCTGTGTATTTT CACTAATACACAGAATATAATTGTAACTCTGCACATTA AAGTTCCAACTGTTTAACACATACTTTGTTGTAGGTC CCCTATCCTTTGACTATGCACATTCCATTGGCAACAA ACCAAAATTAAAATTGAATCCATATTCCTGGACAAAG GTAGGTTTTCGTGTTTGTGTCTTTGTATGTGATCTTTA AAACTATTCAGGTGCGTATGACATTGAGAAGGAAAAT ATCCCAAAACTTTCAGGTTGCCCACATAATATTTTTT AGACGCACCAGGGGGGCACAGGGATTCTCCTTATGCG AAAAAAATTGGGAAAGGATATAGCAATTCTCATGAC

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:

GTGCAAGCCCTACCTCTTTATTACGCTGTAACTCGAG ACGAATTGCTAGGGATGGCGGGAGAGGGTATTTGGTA ATGTTCAATCAGGTGTATTGCGGGTTCGAGTAAATCA CACCTACCCATTGTCTCAAGCAGCACAGGCACACGA AGACCTTGAGAATAGGAAAACATCTGGATCTGTTGTG CTTATCCCCTAAGGCAAATATGAGTTTGGTCTGTTCA TTTTAAAGGACTCGTGGGGGGGTGTGTGAAAATGAAT AAGGAACGTTTGCCTTCCTGTTCCGGATAGCTGCTG TGCCCAATCAAAA

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 - 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; that is 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. Twenty-three 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) showed 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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