Determine Genetic Markers Associated to the Development of Ripening Capacity

Ngoc Nham, Sara Montanari, David Neale, Elizabeth Mitcham
Department of Plant Sciences, University of California, Davis, CA

ABSTRACT

Pear cultivars have different resistance to ripening. The project aims to identify genetic markers associated with the candidate genes involved in the development of ripening capacity. Genetic markers (single-nucleotide polymorphism - SNPs) in regulatory regions and coding regions of all genes available in the database were identified. These markers were also determined in our candidate genes, which were examined in our previous studies and appear to have important roles in regulation of ripening capacity in pear. Future breeding programs could utilize this resource to develop pear cultivars that have the desired ability to ripen at harvest.

INTRODUCTION

Background on Single nucleotide polymorphisms: Single nucleotide polymorphisms (SNPs) are one of the most common types of genetic variation. A SNP represents a difference in a nucleotide - a DNA building block (one nucleotide is replaced with another in a DNA sequence, Figure 1, left). When SNPs occur within a gene or in a regulatory region near a gene, they may play a role in regulating the gene's function (Figure 1, right). SNPs can work as genetic markers to locate genes associated with certain phenotypes (characteristics of species or cultivars, for example: susceptibility to fire blight, ripening capacity).

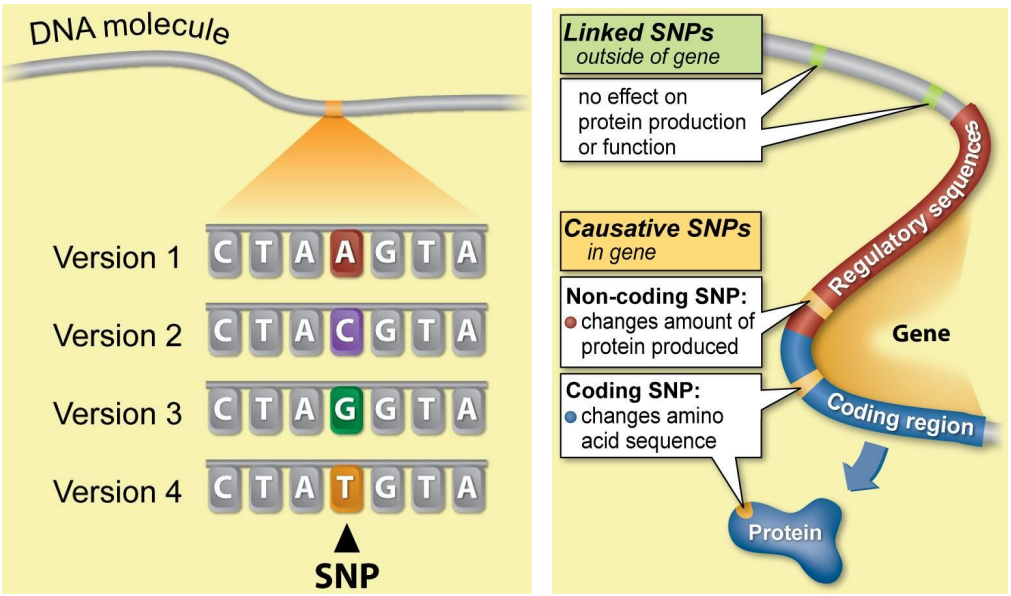

Figure 1: Single nucleotide polymorphisms description (left) and their function in genes (right).

Source: http://learn.genetics.utah.edu/content/precision/snips/
Pear cultivars have different resistance to ripening. Slow ripening at harvest may be desirable if fruit will be stored, but complicates the rapid marketing of pear fruit by requiring conditioning treatments. Some varieties can be very resistant to ripening when first harvested. From our RNA sequencing results and validation experiments conducted over the past few years, we have identified candidate genes that may regulate the development of ripening capacity. The functions of these genes are associated with jasmonic acid metabolism, cold-induced pathways, and transcriptional regulation. In collaboration with David Neale's group, and utilizing his existing genome database of 19 European pear cultivars, the genetic markers (SNPs) associated with the candidate genes are being identified.

OBJECTIVE

1. Identify genetic markers associated with the candidate genes involved in the development of ripening capacity.

PROCEDURES and RESULTS

The description of the experimental procedures is summarized in Figure 1.

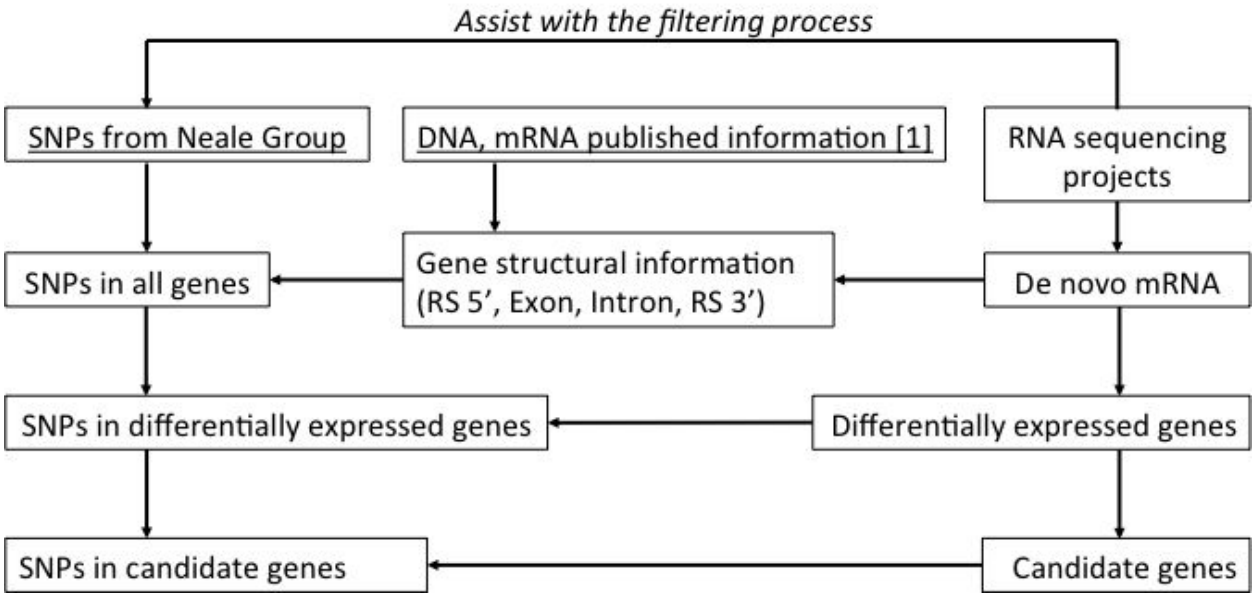

Figure 2: Experimental procedure. The underlined text indicates work that was conducted by other research groups. RS 5', RS 3': regulatory sequence at 5' or at 3' end (please see Fig. 2 for gene structural explanation).
*[1] Chagné D, Crowhurst RN, Pindo M, Thrimawithana A, Deng C, Ireland H, et al. (2014) The Draft Genome Sequence of European Pear (*Pyrus communis *L. 'Bartlett'). PLoS ONE 9(4): e92644. doi:10.1371/journal.pone.0092644*

The genetic markers (SNPs) were identified by Neale's group based on genome sequencing of 55 cultivars. SNPs of 19 European pear cultivars (287,224) were selected for the work in Mitcham's laboratory (Table 1).

Table 1: Numbers (No.) of SNPs and genes

No. SNP (Neale's group)	287,224
No. Genes in the database*	50,691
No. Genes with SNPs	29,331

*database: using predicted mRNA from Chagne et al., 2014 that exist in our de novo mRNAs. This helped to catch the real mRNAs

The *de novo* (computer assembly) mRNA sequences were identified from our previous RNA sequencing project funded by the California Pear Advisory Board. The mRNA (50,691) database was generated based on the sequence comparison between the predicted mRNA published by Chagne et al., 2014 and our *de novo* mRNA sequences (Table 1). Of these genes, 60% (29,331 genes) have SNPs (Table 1).

Based on the position of predicted mRNA and DNA of 'Bartlett' pears (Chagne et al., 2014), we identified Regulatory Sequence, Introns and Exons of these genes (Please see Fig. 2 for description about gene structure). Then, the SNPs in the specific gene areas: Regulatory Sequence, Introns, and Exons were extracted (Table 1, Table 3).

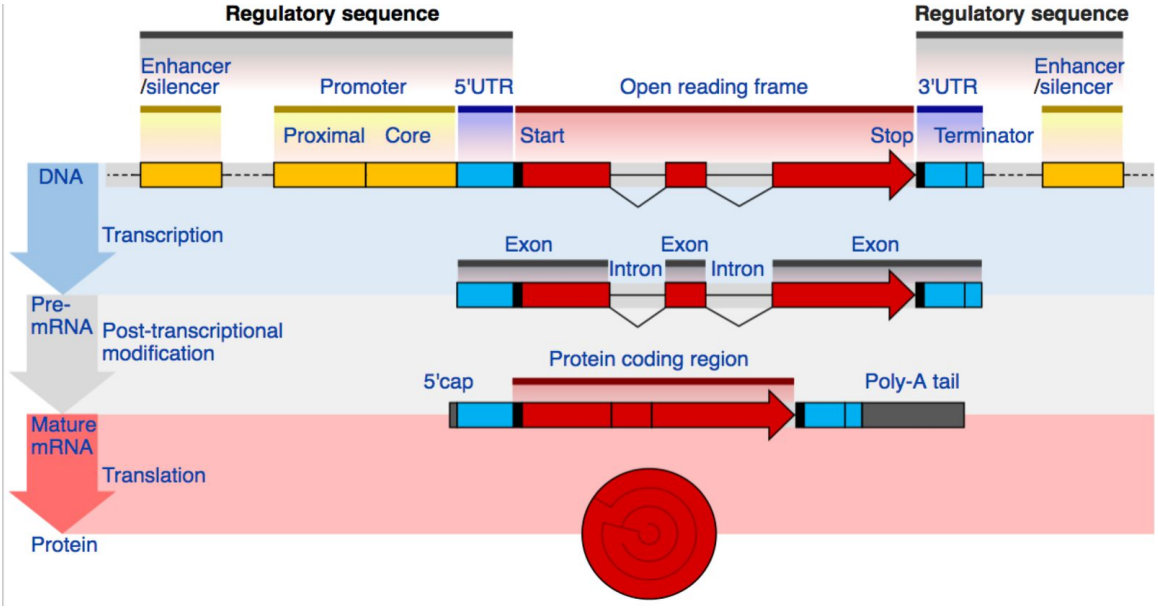

Figure 2: Gene structure. ***Regulatory Sequences*** *and* ***Introns*** *are involved in controlling expression of* ***Exons*** *(coding sequence) to become a protein. Adopted from https://en.wikipedia.org/wiki/Gene.*

Furthermore, based on the results of our previous RNA sequencing project, we found candidate genes that may play an important role in ripening regulation. The functions of these genes are associated with ethylene, jasmonic acid metabolism, cold-induced pathways, and transcriptional regulation. SNPs associated with these genes were also identified (Table 2, 3, 4, 5).

Table 2: Summary of numbers of SNPs in 5' Regulatory Sequence (RS 5'), Exon, Intron, and 3' Regulatory Sequence (RS 3')

	# Genes	# SNPs				
		Total	RS 5'	Exon	Intron	RS 3'
Genes with SNPs	29,331	181,735	12084	79374	81243	9034
All differentially expressed genes	19,268	97,938	5756	42458	45399	4325
Candidate genes	95	410	31	215	142	22

Table 3: Numbers of SNPs in 5' Regulatory Sequence, Exon, Intron, and 3' Regulatory Sequence of candidate genes associated with cell walls

GeneID	RS 5'	Exon	Intron	RS 3'	Putative funtion	Funtional group
AUG2gene00037072.1	0	2	0	0	pectate lyase	cell wall
AUG2gene00013408.1	0	3	2	0	pectate lyase	
AUG2gene00016275.1	0	0	2	0	pectate lyase	
AUG2gene00026349.1	0	3	2	0	pectate lyase	
AUG2gene00026473.1	0	3	1	0	pectate lyase	
AUG2gene00028924.1	0	3	1	1	pectate lyase	

Table 4. Numbers of SNPs in 5’ Regulatory Sequence, Exon, Intron, and 3’ Regulatory Sequence of candidate genes assoc. with transcription factors (major regulator genes).

GeneID	RS 5'	CR	NCR	RS 3'	Putative funtion	Funtional group
AUG2gene00018949.1	0	1	7	0	AP2/ERF	
AUG2gene00019471.1	0	1	0	0	AP2/ERF	
AUG2gene00031596.1	0	1	0	0	AP2/ERF	
AUG2gene00034704.1	0	0	0	2	AP2/ERF	
AUG2gene00023896.1	0	1	0	0	AP2/ERF	
AUG2gene00024719.1	3	3	0	0	AP2/ERF	
AUG2gene00008852.1	0	0	8	1	AGAMOUS-like 24	
AUG2gene00023612.1	0	1	3	0	AGAMOUS-like 24	
AUG2gene00023613.1	0	0	5	0	AGAMOUS-like 24	
AUG2gene00036190.1	1	2	6	0	AGAMOUS-like 24	
AUG2gene00036195.1	6	0	3	1	AGAMOUS-like 24	
AUG2gene00005878.1	1	1	0	0	flowering promoting factor 1	transcription factor
AUG2gene00000984.1	0	1	0	0	flowering promoting factor 1	
AUG2gene00005235.1	0	0	1	1	MYB	
AUG2gene00036776.1	0	1	2	0	MYB	
AUG2gene00025605.1	1	2	3	0	MYB	
AUG2gene00034083.1	0	1	0	0	TCP9a	
AUG2gene00034669.1	0	3	0	0	TCP9a	
AUG2gene00006934.1	0	1	0	0	TCP9a	
AUG2gene00020664.1	0	2	2	0	bHLH	
AUG2gene00021463.1	3	5	1	2	bHLH	
AUG2gene00046955.1	0	2	2	0	bHLH	
AUG2gene00031585.1	0	2	2	0	bZIP	
AUG2gene00003347.1	0	4	6	0	bZIP	
AUG2gene00011735.1	0	1	0	0	HsfB2b	
AUG2gene00001056.1	0	9	9	1	DRE-binding protein	transcription factor and cold-related
AUG2gene00027664.1	0	3	0	0	DRE-binding protein	
AUG2gene00014339.1	2	1	0	1	CBF4	
AUG2gene00010495.1	0	3	3	2	CBF1	

Table 5. Numbers of SNPs in 5' Regulatory Sequence, Exon, Intron, and 3' Regulatory Sequence of candidate genes associated with plant growth regulators.

GeneID	RS 5'	CR	NCR	RS 3'	Putative funtion	Funtional group
AUG2gene00048346.1	0	6	1	0	auxin response factor	
AUG2gene00048602.1	2	4	3	0	auxin response factor	
AUG2gene00010462.1	0	1	4	0	auxin response factor	
AUG2gene00037249.1	0	2	6	0	auxin response factor	
AUG2gene00035752.1	0	10	1	0	auxin response factor	
AUG2gene00013376.1	0	4	1	0	auxin response factor	
AUG2gene00000597.1	0	0	4	0	auxin response factor	auxin
AUG2gene00033853.1	2	3	3	0	auxin responsive protein	
AUG2gene00018910.1	0	2	0	0	IAA inducible	
AUG2gene00039586.1	0	4	0	1	IAA synthase	
AUG2gene00036753.1	0	1	0	0	IAA synthase	
AUG2gene00014212.1	0	3	0	1	IAA synthase	
AUG2gene00016585.1	0	1	0	0	IAA synthase	
AUG2gene00016946.1	0	2	3	0	lipoxygenase	
AUG2gene00027004.1	0	3	4	0	lipoxygenase	
AUG2gene00003418.1	0	1	1	0	lipoxygenase	
AUG2gene00003419.1	0	3	5	0	lipoxygenase	
AUG2gene00003420.1	0	6	2	0	lipoxygenase	
AUG2gene00010801.1	0	1	0	0	allene oxide synthase	jasmonic acid
AUG2gene00020924.1	0	2	0	0	allene oxide synthase	
AUG2gene00002813.1	0	2	1	0	allene oxide cyclase	
AUG2gene00043984.1	1	1	3	0	allene oxide cyclase	
AUG2gene00039160.1	0	1	1	0	allene oxide cyclase	
AUG2gene00046510.1	0	1	0	0	jasmonate-zim-domain	
AUG2gene00014996.1	0	1	1	0	ACO	
AUG2gene00021195.1	0	0	1	0	ACO	
AUG2gene00021196.1	0	4	0	0	ACO	
AUG2gene00018409.1	0	2	0	0	ACO	
AUG2gene00018410.1	0	7	0	0	ACO	
AUG2gene00042324.1	0	7	0	1	ethylene receptor	
AUG2gene00006648.1	0	3	2	0	ethylene receptor	ethylene
AUG2gene00003577.1	0	5	0	1	ETR2	
AUG2gene00019282.1	3	3	1	0	ETR2	
AUG2gene00021237.1	2	15	3	0	ethylene insensitive 3	
AUG2gene00025798.1	1	1	0	0	ethylene insensitive 3	
AUG2gene00039556.1	1	1	0	1	ein3-binding f-box	
AUG2gene00015832.1	0	3	3	0	ein3-binding f-box	
AUG2gene00017985.1	0	5	1	1	ein3-binding f-box	

We also reviewed the literature and found information associated with ripening capacity of 24 European cultivars. However, so far, only SNPs of five cultivars (Bartlett, Coscia, Bosc, Anjou, and Seckel - of 19 European cultivars that were sequenced by Neale's group) are available. After the SNP chip is made (by Affymetrix and Neale's group), SNP information of more pear cultivars will be available (hopefully in one or two months) we will further investigate the association between the SNPs and ripening capacity. We

hope to identify SNPs that are more associated with ripening capacity. After the significance of the SNPs is verified by a breeding population, the SNP can be used in breeding programs to generate fruit with desired ripening capacity.