Almond Board of California
Project Report-2001

Project Title: **Back-up copy of the Molecular Map of Almond x Peach**

Project Leader: Tom Gradziel and Abhaya Dandekar

Cooperating Personnel: P. Martinez-Gomez and S. Arulsekar.

Location: Department of Pomology, University of California at Davis

Objective:

Make backup copies of the plasmid libraries constituting the almond/peach genetic map, and after verifying the trueness-to-genotype of the new library, store them (as an archive) in a protected yet readily accessible form.

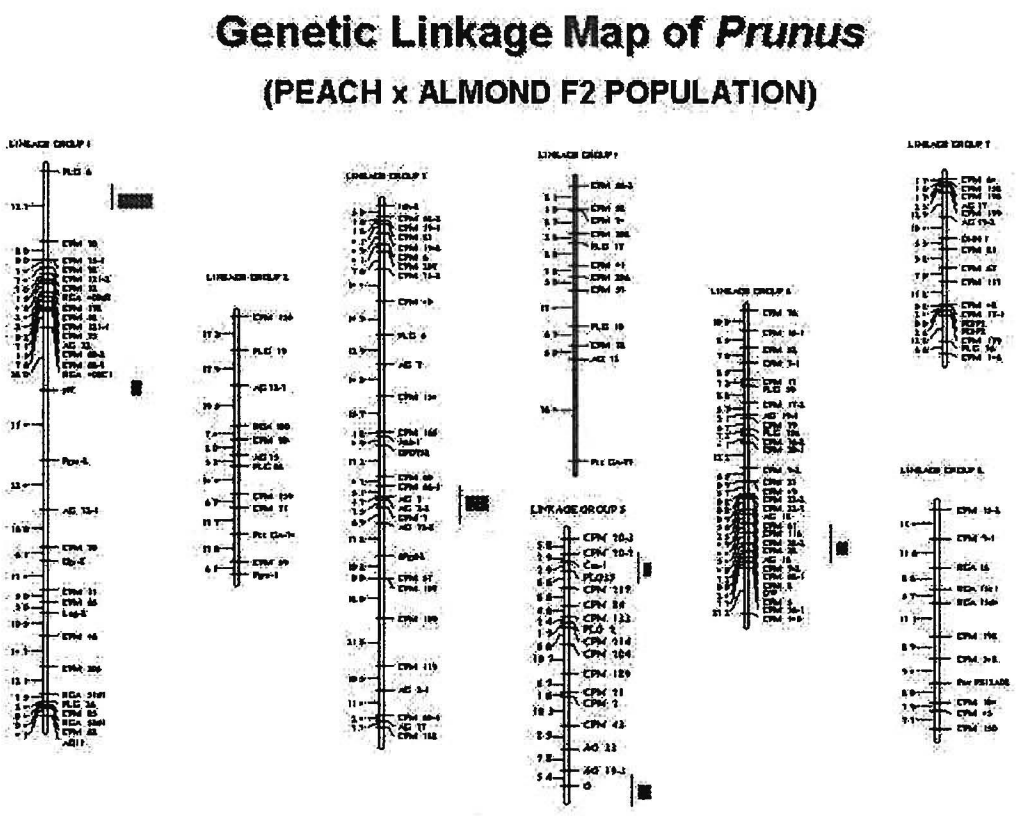
Background and Results:

Over the last 7 years UC Davis has become one of the leading centers of research on plant genomics (that is, the molecular characterization of the genetic makeup of an organism in order to better understand and manipulate its growth, development, response to disease, adverse environments, etc.). An early achievement in this area has been the development of a complete molecular map of the peach and almond genomes by Drs. Bliss and Arulsekar (both now retired). The genetic mapping of peach and almond took several years and cost several hundred thousand dollars, with funding primarily from federal grants and to a lesser extent the California industry. The map was developed based upon peach and almond populations provided by my breeding program, and so are particularly relevant and useful to future improvement of these crops. (This origin and the close genetic similarity between peach and almond make the same map useful to both crops, and this project was jointly funded by the California Cling-Peach Advisory Board and the Almond Board of California). The map consists of a large number of identifiable molecular markers spaced at fairly uniform distances over the chromosomes which contained the DNA and so genes which define the character of the crop species as well as individual variety (Fig. 1). While the markers may not directly code for genes of horticultural importance (for example, productivity, disease resistance, etc.), they "code for" or mark nearby DNA and so allow us to" tag" or mark the chromosomal DNA strand at points close enough to horticultural genes of interest to make them useful. (They are used in much the same way that the page numbers of a book can be used to readily identify and repeatedly locate a sentence or paragraph of interest). By observing which marker is closely inherited with important horticultural traits (such as disease resistance) we can select for that marker

even at the seedling stage and so select for the disease resistance gene even if we do not know its true genetic makeup. In addition, identifying the rough location of the disease resistance gene allows us to closely examine this more focused area of DNA to identify and better characterize the gene. For example, this approach has allowed us to identify the self-incompatibility gene in almond and to developed specific probes or markers to identify each specific cross-incompatibility group. While this has great value to the breeding program for planning new crosses, it has also had direct value to the growers as they now have rapid and accurate identification of the cross incompatibilities of different California almond varieties.

The individual molecular markers that comprise the map have previously been stored as small circular segments of DNA called plasmids as these allow their rapid increase when inserted into *E. coli* bacteria. (Bacteria normally store their DNA as circular plasmids and so can naturally increased plasmid numbers as the bacteria divide and multiply). Such libraries can be easily lost, however, either by direct loss of the bacterial populations, or by contamination. Because the peach and almond library existed as only a single copy, it was particularly vulnerable to loss or contamination. In this project, back-up copies of the library have been made to serve as a reserve copy in case the original is lost and to serve as the basis for future research. The back-up copy has been archived as both an *E. coli* plasmid preparation and as sequences of the individual marker DNAs.

Fig. 1.



Ninety-one cDNA clones were identified from frozen stocks of clones isolated from the original peach-almond cDNA library. Bacterial cultures were initiated from the frozen stocks that were in tubes segregated in three different boxes. The objective was to both back-up and identify /discriminate among these clones to segregate the unique clones that can be used for mapping fruit traits in almonds or peaches. Part of the characterization involved DNA sequence analysis that can then be used as a unique sequence Tag for each clone. Additionally, gene annotation programs to find homology and to assign putative function evaluated the DNA sequence information. In order to accomplish these tasks the inoculated cultures were archived on 96 well dishes (Table 1). This was done in the newly established CA&ES Genomics facility. The archived plate was used to inoculate a fresh deep 96 well dish. The inoculated cultures were grown overnight at 37C in a high grow shaker. Cultures were then harvested to isolate the bacteria and plasmid DNAs were extracted using a liquid handling robot. Purified plasmid DNA templates were used for cycle sequencing in one direction using an internal plasmid primer (external to the insert cDNA). DNA sequences have now been obtained for all 91 clones. The DNA sequence data are now being analyzed further. While the bacterial cultures provide back-up copies of the original mapping library, the DNA sequence data provide an additional and more readily available characterization of the marker DNA. This sequence data also allow a fuller characterization of marker DNA (as presented in Table 1) and allows an initiation point for exploring new genomic approaches for processing peach improvement.

Table 1: DNA sequence analysis of marker clones and proposed function based on BLAST analysis.

S. No	Position in Dish	Clone Designation	ORFs	BLAST >80	BLAST 30-80	BLAST <30	Function
1	A1	PCPMA1	1			NOSH	Unknown
2	A2	PCPMA2	4			laccase	
3	A3	PCPMA3	2			cystinosin	
4	A4	PCPM4	2			NADH dehydrogenase	
5	A5	PCPM5	4	allergen			
6	A6	PCPM6	5			Gda-1 protein	
7	A7	PCPM7	2		lacZ		
8	A8	PCPM8	2			NOSH	Unknown
9	A9	PCPM9	2			Cps export prot	
10	A10	PCPM10	1			NOSH	Unknown
11	A11	PCPM11	3			Helicase	
12	A12	PCPM12	2		extensin		Cell Wall
13	B1	PCPM13	11	HPRGP			Cell Wall
14	B2	PCPM14	5		lacz		
15	B3	PCPM15	5			Fca/m receptor	
16	B4	PCPM16	5	RNA binding prot			
17	B5	PCPM17	6		lacz		

18	B6	PCPM18	6	L2 ribosomal prot			
19	B7	PCPM19	6	aquaporin			
20	B8	PCPM20	4		lactamase		
21	B9	PCPM21	4		lacz		
22	B10	PCPM22	1			NOSH	Unknown
23	B11	PCPM23	6		lacz		
24	B12	PCPM24	6	aquaporin			
25	C1	PCPM25	4		lactamase		
26	C2	PCPM26	3			NOSH	Unknown
27	C3	PCPM27	1			NOSH	Unknown
28	C4	PCPM28	2	Triose P isomerase			
29	C5	PCPM29	7	c-myc binding prot			
30	C6	PCPM30	4	Steroid binding prot			
31	C7	PCPM31	3		Gluc 6p isomerase		
32	C8	PCPM32	7			DNA binding prot	
33	C9	PCPM33	3			NOSH	Unknown
34	C10	PCPM34	3			ADP ribosyl transferase	
35	C11	PCPM35	5			Heterodisulfide reductase	
36	C12	PCPM36	5		lacz		
37	D1	PCPM37					
38	D2	PCPM38					
39	D3	PCPM39					
40	D4	PCPM40					
41	D5	PCPM41					
42	D6	PCPM42					
43	D7	PCPM43					
44	D8	PCPM44					
45	D9	PCPM45					
46	D10	PCPM46					
47	D11	PCPM47					
48	D12	PCPM48					
49	E1	PCPM49	3	Gast like prot			
50	E2	PCPM50	2			Cg3424	
51	E3	PCPM51	4		Pectate lyase		
52	E4	PCPM52	6		Seed protein		
53	E5	PCPM53	3		Zinc finger		
54	E6	PCPM54	8		catalase		
55	E7	PCPM55	1			cyclin	
56	E8	PCPM56	5		Allergen protein		
57	E9	PCPM57	2			NADH Dehydrogenase	
58	E10	PCPM58	3			Histone deacetylase	

59	E11	PCPM59	3		CLP-protease		
60	E12	PCPM60	5			lacZ	
61	F1	PCPM	3	Wound stress induced protein			
62	F2	PCPM	1				
63	F3	PCPM	3	Calmodulin			
64	F4						
65	F5						
66	F6						
67	F7						
68	F8						
69	F9						
70	F10						
71	F11						
72	F12						
73	G1						
74	G2						
75	G3						
76	G4						
77	G5						
78	G6						
79	G7						
80	G8						
81	G9						
82	G10						
83	G11						
84	G12						
85	H1						
86	H2						
87	H3						
88	H4						
89	H5						
90	H6						
91	H7						
92	H8						
93	H9						
94	H10						
95	H11						
96	H12						