FINAL PROJECT REPORT

Project Title: Inheritance of chilling-dependent pear fruit ripening

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Cooperators: None

Other funding sources: None

Total Project Funding: $10,938

Budget History:

Item	2013
Salaries	
Benefits	
Wages	$8,202
Benefits	$ 656
Equipment	
Supplies	$2,080
Travel	
Plot Fees	
Miscellaneous	
Total	**$10,938**

ORIGINAL OBJECTIVES

1. Determine the segregation of an absolute and quantitative requirements for fruit chilling in the two crosses by evaluating fruit ripening after 7 days at 20°C following 0, 30, and 60 days at -1°C.
2. Determine the genetic composition of the parents and selected seedlings with no, moderate, and high chilling requirements for allelic variants of ACS genes, and determine the relationship between genotype and chilling-requirement.

SIGNIFICANT FINDINGS

- Seedlings segregated for chilling-independence (n=19) and chilling-dependence (n=13) for fruit ripening. This ratio, tested by chi-square analysis, is consistent with genetic control by a single gene or genetic locus.
- There were no genetic differences among the parents and the seedlings for the ACS1 and ACS2 using an agarose gel detection system and the ACS1 and ACS2 allele-specific primers reported by El-Sharkawy et al. (2004). These results were duplicated in the laboratory of Nahla Bassil at the USDA, ARS, National Clonal Germplasm Repository in Corvallis, Oregon. Dr. Bassil also tried genotyping an additional 7 cultivars and breeding selections, including 'Passa Crassane' and 'Old Home', which El-Sharkawy reported were chilling-dependent and chilling-independent, and which he reported to differ in their ACS2 genotypes. She did not find any differences. These results are also consistent with results obtained by Dr. Amit Dhingra of Washington State University. However, using a capillary electrophoresis system, Dr. Bassil detected a small fragment (81 base pairs) of ACS1b which may be associated with chilling-dependence.

RESULTS AND DISCUSSION

In 2013, the presence or absence of a chilling requirement for pear fruit ripening was determined for 32 seedlings of the cross 'Beurre d'Anjou' (high chilling requirement) and US76128-009 (low chilling requirement). Seedlings segregated for chilling-independence (n=19) and chilling-dependence (n=13) (Table 1). This ratio is statistically consistent with a 1:1 segregation indicating single gene control. With data from 2011, a total of 45 seedlings have been evaluated. Among those seedlings which exhibited chilling-dependence, five required 4 weeks of chilling, three required 8 weeks of chilling, and five did not ripen after 8 weeks of chilling, and apparently require more than 8 weeks. The chilling requirement of an additional five could not be accurately determined due to insufficient fruit for all treatments. The genetic control of quantitative difference in chilling requirement could not be determined from this type of analysis, but is probably due to different genes than that controlling the chilling dependence versus independence.

No differences in ACS1 and ACS2 genotypes were detected using an agarose gel detection system and the ACS1 a/b and ACS2 a/b allele-specific primers reported by El-Sharkawy et al (2004) (Table 2). We concluded that new primers designed from the ACS1 and ACS2 gene and/or promoter sequences are needed to genotype pears for these genes involved in ethylene biosynthesis and to answer the question of whether they control fruit chilling response in a qualitative or quantitative manner. Similar results were obtained in the laboratory of Dr. Nahla Bassil, who collaborated on the study. The DACS1a primer amplified a larger fragment than reported by El-Sharkawy et al. (2004). The primers for DACS1b, DACCS2a, and DACS2b amplified products similar to those reported by El-Sharkawy, but no polymorphisms were observed in 'Old Home' and 'Passa Crassane', the two pear cultivars used in his study, nor in seven other pear cultivars which varied in date of maturity, and presumably in chilling requirement (Table 3). However, polymorphic products of low molecular weight were found with the DACS1b (73 bp, 75 bp, 79 bp and 81 bp) and DACS2b (109 bp and 115

bp) primers were found (Table 4). The 81 bp DACS1b fragment was associated with those cultivars and selections which had a chilling requirement. The significance of this fragment must be verified with additional cultivars, selections and segregating seedling populations of known chilling requirement.

REFERENCES

El-Sharkawy, I., B. Jones, L. Gentzbittel, J.-M. Lelièvre, J. C. Pech and A. Latché. 2004. Differential regulation of ACC synthase genes in cold-dependent and –independent ripening in pear fruit. Plant Cell and Environment 27:1197-1210.

Table 1. Segregation of chilling-independence versus chilling-dependence[1].

	Chilling-Dependent		
Chilling-Independent	4 weeks	8 weeks	8 weeks+
19	5	2	6
Total 19		13	

[1] Chi-square analysis detected no deviation from the hypothesized 1:1 ratio (Pr> ChiSq = 0.37).

Table 2. Amplified fragment sizes (in number of DNA base pairs) for 'Old Home' and 'Passe Crassane' (Experiment 1)

	ACS gene and allele			
Cultivar	ACS1a	ACS1b	ACS2a	ACS2b
Old Home	~900	405	266	437
Passe Crassane	~900	405	266	437

Table 3. Amplified fragment sizes (in number of DNA base pairs) for eight pear cultivars (Experiment 2).

			DACS1		DACS2	
CPYR Number	and Cultivar	Maturity	Expected	Observed	Expected	Observed
1057	Angelica di Saonara	Early	785/405	405	437	437/266
1165	Buerre Bosc	Late	785	405	266	437/266
148	Abate Fetel	Intermediate	785/405	405	437/266	437/266
77	Doyenne du Comice	Late	785	405	266	437/266
448	Pautalia	Early	785/405	405	437	437/266
431	Old Home	Intermediate	785/405	405	437/266	437
441	Passe Crassane	Late	785	405	266	437

Table 4. Amplified fragments sizes based on capillary electrophoresis using M13-tagged primers.

		DACS1b		DACS2a		DACS2b	
CPYR	Number and Cultivar	Expected	Observed	Expected	Observed	Expected	Observed
441	Passe Crassane	421	73, 81	266	317	489	109
63	Anjou	421	79, 81	266	317	489	109
431	Old Home	421	79, 81	266	317	489	109, 115
	US78307-045	421	79, 81	266	317	489	109
	US84907-166	421	75, 79	266	317	489	109
	US76128-009	421	75, 79, 81	266	317	489	109
	Sunrise	421	79	266	317	489	109
	NJ B9 R1 T117	421	75, 79	266	317	489	109

EXECUTIVE SUMMARY

Winter pears, such as 'Beurré d'Anjou', 'Buerré Bosc' and Doyenné du Comice', require a period of cold storage and/ or ethylene exposure to initiate normal ripening. The inability to ripen, while perhaps contributing to long storage life, poses challenges to the pear industry in their ability to deliver a ready-to –eat product to market. The development of new cultivars with which are more amendable to post-harvest manipulation requires knowledge of the inheritance of the post-harvest chilling requirement. In addition, knowledge of the underlying genomic basis for the trait and the genetic composition of important cultivars and breeding parents will improve the ability to breeding new cultivars with desirable post-harvest ripening. Preliminary data for a seedling population derived from hybridizing 'd' Anjou' (chilling –dependent with long requirement) and US76128-009, a chilling-dependent selection with a short requirement) suggested that chilling-dependence is a dominant trait, and that the amount of chilling is variable, likely determined by an unknown number of genes. The purpose of this study was to determine the inheritance of the absolute requirement for chilling as well as to characterize the nature of the quantitative variation in chilling requirement.

Summary of findings

Approximately half of the 45 seedlings exhibited chilling independence for fruit ripening, while the others exhibited chilling dependence, with the amount of chilling required varied from 4 to 8 weeks or more. This finding is consistent with genetic control by a single gene. It had been hypothesized that the genes ACS1 and ACS2 controlled chilling-dependence. Using gene primers published by El-Sharkawy, we found no relationship of these genes with chilling dependence/independence. We were unable to duplicate El-Sharkawy's results with the cultivar he used in his study, 'Old Home' and 'Passe Crassane'. We hypothesized that different primers designed from the gene sequences would be necessary to further investigate the relationship. Subsequent communication from other researchers confirmed that these genes do not control chilling dependence/independence. We did find a possible relationship of a small DNA fragment (81 base pairs) with chilling dependence.

Future directions

Evaluation of additional seedlings would enhance the robustness of the analysis of the genetic control of chilling dependence, especially the quantitative variability in the amount of chilling needed to initiate normal ripening. We plan to do that in the growing season. In addition, new seedlings of the cross between 'Anjou' and 'Harrow Delight', which has not chilling requirement, will be generated by hybridization. We plan to determine the relationship between genotype, global gene expression patterns, and chilling-requirement using advanced genomic approaches, Pnome and RNA-Seq. We will validate the association of the 81 bp fragment with chilling independence in additional pear cultivars and seedlings. The overall goal will be to develop new primers or markers designed based on the gene sequences derived from the European pear genome sequence.