FINAL PROJECT REPORT

Project Title: Genetic analysis of advanced pear rootstock

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Total Project Funding: **Year 1:** 49,362

Other funding sources – none

Budget 1

Organization Name: WSU **Contract Administrator:** Carrie Johnston

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Item	2013
Salaries	
Benefits	
Wages[a]	36,510
Benefits	2,352
Equipment	
Supplies[b]	10,000
Travel	1,500
Miscellaneous	
Plot Fees	
Total	49,362

Footnotes:

a. Wages to partially support the time of a graduate student and an undergraduate student on the project

b. DNA quantification, quality analysis, PCR, comprehensive genetic analysis and subsequent family structure analysis - ~250 samples

OBJECTIVES

The objective of this 1 year project was to determine the genetic relationships amongst the ad vanced and elite pear rootstock germplasm available outside of the USA which we plan to im port over the next few years to jump start pear rootstock breeding.

Even before the plant material is imported via Prosser or USDA Beltsville, there is a need to understand the genetic diversity of the material. This will enable the prioritization of the indi viduals to be imported and the order in which they should be imported. The aim is to import r epresentatives of most diverse selections to have the largest degree of genetic diversity.

An additional bonus of the genetic analysis prior to the introduction of the germplasm throug h the CPC is that there will be a baseline fingerprint already available that can then be utilize d to confirm trueness-to-type at a later date. This is critical as exemplified with the recent gen etic analysis of OHxF87 where it has been found that Bartlett and not Farmingdale is the mal e parent. There are several other examples of mix ups recently with the Geneva rootstocks. T herefore, utilization of a systematic approach was proposed to be employed in this project.

Objective 1: Genetic diversity analysis of advanced rootstock selections obtained from collab orating pear breeding programs. (Year 1)

Specific Goals:

a. Perform DNA marker analyses for ~250 individuals, score markers using a binary c ode (0, 1)
b. Convert information into a unique genetic identifier and a barcode
c. Perform population structure analysis to identify genetic relationships among indivi duals
d. Submit the data and results to GDR-Database for Rosaceae

SIGNIFICANT FINDINGS

- Population structure analysis identified 3 main populations amongst the DNA samples representing 29 advanced rootstocks obtained from University of Bologna.
- Grouping of individuals does not correspond to the indicated parental descent implying potential mixing of inventory.
- The DNA marker analysis method produces substantial information for a robust population analysis.

RESULTS AND DISCUSSION

In order to begin DNA marker analyses, a primer screen was performed to identify primer co mbinations that would amplify the greatest number of polymorphic loci that would be most i nformative about population structure since there are no previous reports of application of Ta rgeted Region Amplified Polymorphism (TRAP) markers in pear. A total of 48 primer combi nations were tested with 39 primer combinations, each amplifying between ~20 to ~75 loci fr om a single genotype were identified through this primer screen.

Perform marker analyses for ~250 individuals:
Primers were selected based upon the number of loci amplified in the primer screen. Three p rimer pairs (six total combinations) were used for PCR. DNA for 34 pear samples was receiv ed from Stefano Musacchi at University of Bologna. PCR was performed in triplicate for eac h of these samples. PCR products were subsequently visualized on a polyacrylamide gel. Gel images were scored for each individual using a binary system of 0 or 1, indicating absence or presence of a marker, respectively. Results were recorded in an Excel spreadsheet for analysi s.

Conversion of information into a unique genetic identifier and barcode:
The custom software seeDNA© developed by the Dhingra lab was used to simultaneously ge nerate a unique genetic identity (GID) code, a scannable barcode, and two dimensional gel i mage for each individual. Each two dimensional gel image and barcode contains all of the sc oring information obtained from the PCR. Each color in the 2D gel represents a different pri mer combination (these are indicated by arrows on Figure 1A). The lengths of the horizontal lines correspond to the molecular weight of that particular marker. This information is unique to the individual from which the DNA was taken. With that in mind, the images shown in Fi gure 1B can be used as ID tags in nurseries or by growers in the field. Scannable barcodes an d QR codes allow for easy access of information regarding the individual. In addition to funct ioning as an ID tag to aid in inventory management, the information on this tag can be used t o reveal potentially mistaken identity based on mislabeling. To accomplish this, DNA from t he unknown sample can be analyzed and compared to the marker scoring information from a sample.

seeDNA© was also used to compare similarity between individuals. Figure 1A shows sampl es that are 83% identical to the pear sample number 26 (1B), an open pollinated cross. Sampl e 26 is shown on the far left while the two most similar relatives are depicted in the middle a nd right of 1A. Note that although there are a significant amount of similarities between the t hree gel images there are several markers that differ between these individuals. Information s uch as this may be helpful in identifying potential parental redundancy between these individ uals. The results from seeDNA© are in agreement with those from both STRUCTURE and N TSys.

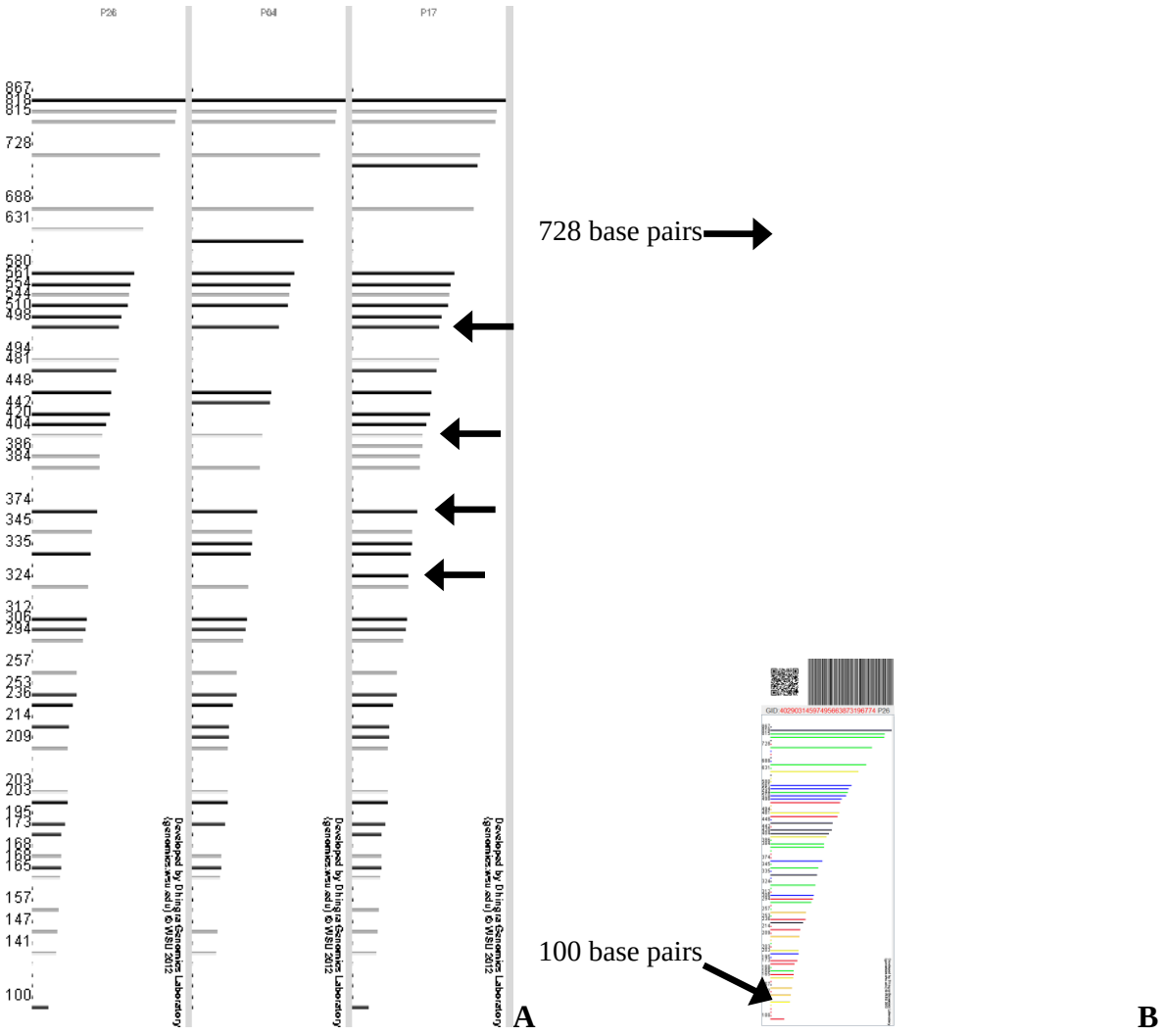
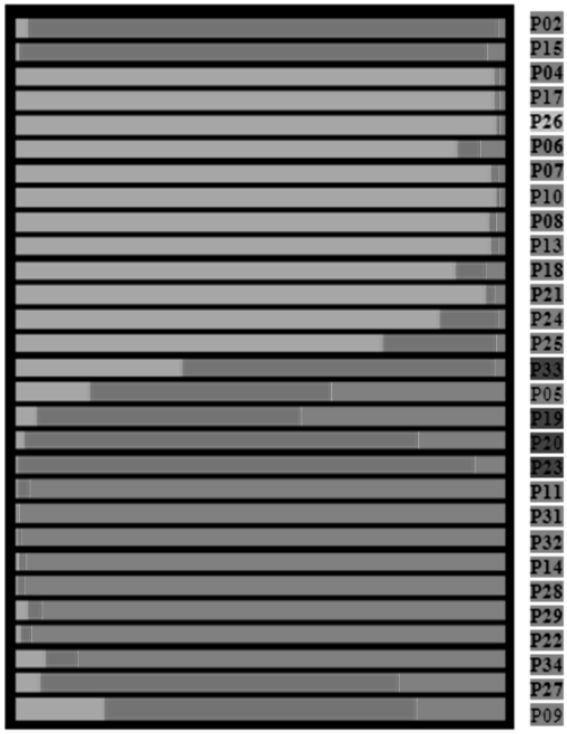


Figure 1: seeDNA© generated output. Comparison of individuals 83% similar to sample nu mber 26 (A) and identification tag containing genetic identification (GID) code, sample name , two dimensional gel image, and scannable barcode (B).

Population structure analysis:
The polymorphic loci information was used for population structure analysis. TRAP primers produced 86 polymorphic markers among the 29 of the 34 pear samples. We were unable to obtain sufficient amplification of markers for 5 samples; DNA for these samples has been req uested to complete the analysis. The program STRUCTURE was used to assign individuals i nto populations based on probability and the presence or absence of a marker, i.e. genotype d ata. Results were also analyzed by the program NTSys which creates a phylogenetic tree usin g statistical algorithms and a specified similarity coefficient.

The genetic relationships among these individuals were assessed using STRUCTURE and N TSys. STRUCTURE output is shown (Figure 3) as horizontal bars, each bar representing a si ngle individual. Within each horizontal bar are colored portions, each color is one of the pop ulations determined by the program. The proportion of a color with a horizontal bar indicated

the proportion of genes from that individual that belong to that particular population. For exa mple samples 11, 31, 32, 14, 28, 29, 22, 34, and 27 have a large proportion of genes that belo ng to the yellow population. When additional information is factored in to these results, we s ee that these individuals are all crosses from the same two parents Abate Fetel x sel. 7950407 4. Other information regarding desirable traits, location, and growth habits can be factored in to identify even more correlations between these individuals and populations. Relationships b etween these individuals become more identifiable when this output is paired with the phylog enetic tree produced by NTSys. Figures 3 and 4 can be laid side by side to show the populati ons (or families) and the admixture within each of the individuals in those families.



Cross Information by Color
US309 x NIJISSEIKI
Passa Crassana x Decana
Abate x California
Abate x sel. 79504074
Abate x M.R. Bartlett
Abate x Rosired
Decana x M.R. Bartlett
Open pollination
Butirra Hardy x Kaiser

Figure 3: Output from the program STRUCTURE indicating the proportion of an individual (a row) that belongs to a particular population (a color: blue, orange, or yellow) as determine d by the program. Sample numbers are listed to the right of each row and are color coordinate d according to the parental cross.

NTSys was useful in identifying relationships among the pear individuals analyzed. This phy logenetic tree can be used to discern similarity between individuals. Returning to the sample number 26 example, you can see that the two most related samples to 26 are numbers 4 and 1 7. While these individuals may have desired traits on their own, it would be at the discretion of the breeder whether it would be beneficial to make a cross between such genetically simila r individuals. Among the individuals analyzed thus far are several progeny of Passa Crassana (Passe Crassane in French). Passa Crassana is an ideal parent as it has compact habits and is frost resistant (USDA-ARS-NGRP). The Asian pear Nijisseiki, another suitable parent, produ ces a medium size tree and is only moderately susceptible to fire blight.

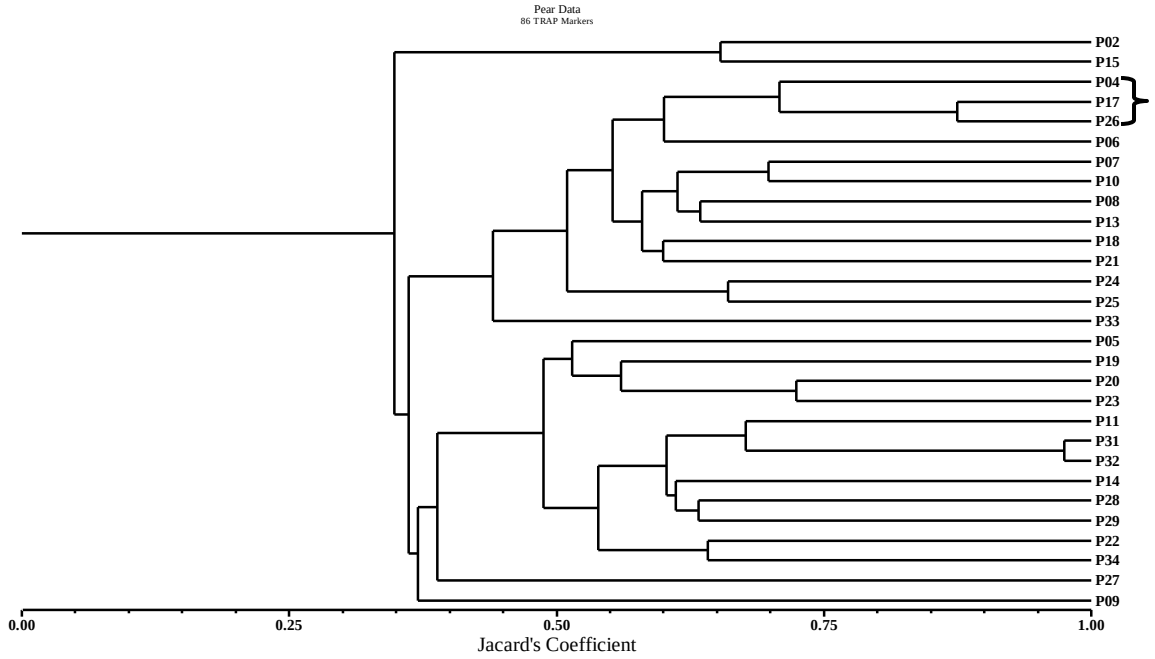


Figure 4: Phylogenetic tree created using the program NTSys. Samples are in the same verti cal order as the STRUCTURE output so as to facilitate side by side comparison.

We have determined that these methods are successful in identifying genetic relationships am ong different species of *Pyrus.* Progress on this project has shown that there is a significant a mount of genetic diversity available in advanced and elite pear rootstock material from Italy. DNA from additional samples will be received in Feb-March 2014. With the addition of the material, we will have a broad understanding of the genetic relationships among these advanc ed selections. This knowledge will assist breeders in making more informed crosses to create dwarfing and disease resistant rootstocks.

The information generated through this project will be submitted to GDR. This will serve as a catalog of genetic information about the imported individuals. As more genomic informatio n becomes available for pears, we can begin to link marker information to traits of interest. We have established a valuable tool for pear rootstock and variety breeding.

Once all the samples are analyzed in 2014 an update shall be provided to WTFRC.

Literature cited

USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Netw ork - (GRIN)*. [Online Database] National Germplasm Resources Laboratory, Beltsville, Mar yland. Available: http://www.ars-grin.gov/cgi-bin/npgs/acc/search.pl?accid=%20PI+131662 (23 January 2014)

USDA, ARS, National Genetic Resources Program. *Germplasm Resources Information Netw ork - (GRIN)*. [Online Database] National Germplasm Resources Laboratory, Beltsville, Mar yland. Available: http://www.ars-grin.gov/cgi-bin/npgs/acc/display.pl?1180597 (23 January 2014)

Executive Summary

Significant progress
Utilizing gene-based markers, we have cataloged the elite rootstock selections from University of Bologna into 3 major sub-populations. This will guide what rootstocks need to be imported and in what order. The information has been provided to Dr. Stefano Musacchi who shall now combine this information with agronomic traits and make a decision on the import process.

Summary of findings
Targeted Region Amplified Polymorphism type of markers generated suitable amount of polymorphic loci to resolve population structures. It was expected that the siblings derived from listed parents shall group together. However, based on information provided by Dr. Musacchi, the parents of the populations were not controlled so outcrossing is possible. This analysis has resulted in the proper identification of the parents as well.

Future directions
Successful resolution of population structure with the markers used for 29 samples will be utilized for the remaining samples. This information will be utilized in the future selection of parental material for pear rootstock breeding.