Detection of Cell Wall Chemical Variation in *Zea mays* Mutants Using Near-infrared Spectroscopy

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Abstract

Detection of Cell Wall Chemical Variation in *Zea mays* Mutants Using Near-infrared Spectroscopy. NICOLE BUYCK (Colorado State University, Fort Collins, Colorado 80521), Steven Thomas (National Renewable Energy Laboratory, Golden, Colorado, 80401).

Corn stover is regarded as the prime candidate feedstock material for commercial biomass conversion in the United States. Variations in chemical composition of *Zea mays* cell walls can obviously affect biomass conversion process yields and economics. Mutant lines were constructed by activating a Mu transposon system. The cell wall chemical composition of 48 mutant families was characterized using near-infrared (NIR) spectroscopy. NIR data were analyzed using a multivariate statistical analysis technique called Principal Component Analysis (PCA). PCA of the NIR data from 349 maize leaf samples reveals 57 individuals as outliers on one or more of six Principal Components (PCs) at the 95% confidence interval. Of these, 19 individuals from 16 families are outliers on either PC3 (9% of the variation) or PC6 (1% of the variation), the two PCs that contain information about cell wall polymers. Those individuals for which altered cell wall chemistry is confirmed with wet chemical analysis will then be subjected to fermentation analysis to determine whether or not biomass conversion process kinetics, yields and/or economics are significantly affected. Those mutants that provide indications for a decrease in process cost will be pursued further to identify the gene(s) responsible for the observed changes in cell wall composition and associated changes in process economics. These genes will eventually be incorporated into maize breeding programs directed at the development of a truly dual use crop.

Introduction

Corn stover (the stalks and leaves of *Zea mays*) is regarded as the prime candidate feedstock material for commercial biomass conversion in the United States. Although environmental changes can influence genetic read-out, a large component of the variability in the chemical composition of corn stover can be explained by the genetics of the individual. For example, previous work with brown midrib maize (*Z. mays*) varieties indicates lower lignin composition (Barrière and Argillier, 1994). Variations in chemical composition of plant cell walls can obviously affect biomass conversion process yields and economics. This study will increase the understanding of the extent of genetic variability in cell wall composition of *Zea mays* and its effect on biomass conversion.

Previous efforts to screen a portion of a mutant library of maize yielded 106 candidate mutant pools from a collection of approximately 5000 individuals screened. The 5000 individuals represented 244 pools of 12 mutant families each (2928 mutant families). Mutant lines were constructed by activating a Mu transposon system which causes the Mu genetic element to move from one location in a chromosome and re-insert itself into another location (transposition). Of the 106 candidates, four pools of mutants were deconvoluted and re-grown as 48 separate families during the winter of 1999-2000. A total of 349 mature, green plants were harvested from these families and shipped to the National Renewable Energy Laboratory (NREL) in Golden, Colorado for further analysis. Characterization of these plants using near-infrared (NIR) spectroscopy is the subject of this study.

Screening of maize plants from these 48 families with NIR spectroscopy has identified 57 individuals as chemical outliers in one or more of the six Principal

Components produced in the analysis. The individuals were identified as outliers relative to a normally distributed population at the 95% confidence level. Of these, 19 individuals from 16 families are described by the two PCs that describe variance in cell wall polymers. These 16 families will be subjected to further analysis.

Materials and Methods

Zea mays plants were grown in Hawaii, under contract to Cold Spring Harbor Laboratory (CSHL), using standard cultivation methods during the winter of 1999-2000. Twelve sibling seeds from 48 mutant maize families, belonging to four previously identified chemical outlier pools, were planted and grown to maturity. Above ground portions of whole plants were harvested at maturity while still green, and stored in breathable polypropylene onion bags with a paper tag identifying the family. Plants were shipped via overnight express to the National Renewable Energy Laboratory (NREL) and upon arrival the plants were dried at 50 ^{o}C for 48 hours. Dried plants were stored at room temperature for approximately 2 months before processing for near-infrared (NIR) spectroscopy.

Each of the mutant lines is genetically segregating for each transposon insertion event in the genome (~200 Mu insertions per genome). Mutations caused by Mu insertions usually give rise to recessive Mendelian phenotypes, therefore only one quarter of the individuals in a family are expected to display a given mutant phenotype. Thus, multiple individuals per family are needed in order to detect mutant phenotypes, in this case altered chemical composition of leaf tissue. Because we obtained several individuals per family, each plant was assigned a unique identification number,

corresponding to the Hawaii mutant code and the plant number from that family, numbered consecutively from 1 (e.g., 764-1).

The leaves from each individual were harvested, excluding the flag leaves located just below the tassel and the juvenile leaves located at the base of the stalk. Only the leaf blades (from the distal leaf tip to the ligule and including the midrib) were harvested and uniquely labeled. The leaf sheath was not harvested.

It was determined that data collection is best performed after the plants have been coarsely ground. Thus, each bag of leaves was fragmented using a kitchen appliance (Braun, models MR430HC and MR370). Although particle size varied, (ranging from a fine powder to 10 millimeters in diameter) the average dimension was approximately 2 millimeters across.

NIR spectroscopy (FieldSpec Pro, model No. FSP350-2500P; Analytical Spectra Devices, Inc., Boulder, CO) data were collected for the ground maize leaves with the probe located 4.5 cm above the sample, yielding a circular 2 cm field of view. A white light source was located 36 cm above the samples, at an angle of 60 degrees from vertical. Leaf samples were placed in an ordinary soda bottle-cap and placed beneath the probe for analysis. Thirty spectra were collected and averaged for use in statistical analysis of the results.

Data were analyzed using a multivariate statistical analysis technique called Principal Component Analysis (PCA) (Meglen, 1992). PCA was accomplished using computer software entitled “The Unscrambler” (by Camo Software, P.C. version 7.5).

Results

PCA of the NIR data from 349 maize leaf samples reveals 57 individuals as outliers on one or more of six Principal Components (PCs) at the 95% confidence interval. Of these, 19 individuals from 16 families are outliers on either PC3 or PC6, the two PCs that contain information about cell wall polymers. PC1 and PC2 explained 68% and 18% of the variance, respectively. The PC-loading suggests that PC1 accounts for differences in the brown color of the samples (Figure 1). We suspect that this is largely due to the degree to which the plants were hosting a surface fungal colonization. PC2 explains differences in density (signal intensity) among the samples (Figure 2). These components are not pertinent to this study and will not be discussed further.

The remaining four PCs contain chemical information that may be of value from the point of view of biomass conversion. PC3 explains 9% of the variation among all the samples and the PC-loading suggests this variation is due to a combination of chlorophyll and cellulose content (Figure 3). PC4 suggests that 3% of the variance among all the samples can be explained by the chlorophyll content alone (Figure 4). The PC-loading for PC5 suggests that 1% of the variance can be explained by the chlorophyll and carotenoid content of the samples (Figure 5). Lastly, PC6 explains less than 1% of the variance among all the samples and the PC-loading plot suggests this is due to a combination of chlorophyll, carotenoid, and sugar content (Figure 6). Chlorophyll (green) and carotenoids (yellow, orange and red) are common leaf pigments visible to the human eye.

Of the six Principal Components, PC3 and PC6 contain information relevant to plant cell wall polymers important for biomass conversion. Scores for PC3 and PC6

were plotted for each individual sample (PC3 versus PC6), and the plot identifies 22 outliers at the 95% confidence level (Figure 7).

Discussion and Conclusions

The 22 outliers identified in Figure 7, correspond to 19 individuals from 16 families. These 22 individuals are listed in Table 1. Three of the outliers are replicates of the same sample (family 633). In Table 1 there are three cases where two individuals from the same family (boldface type) are listed as outliers. These three families (624, 758 and 762) are important because the presence of two outlier individuals in the same family lends greater credence to the hypothesis that the family has unusual cell wall chemistry caused by a genetic mutation. In particular, it should be noted that family 624 shows 2 outliers out of 7 total plants, family 758 shows 2 outliers out of 9 total plants, and family 762 shows 2 outliers out of 7 total plants screened. In each case, the number of outliers approximates one quarter of the individuals screened from each family, lending further credence to the genetic source of the variability detected. These three families shall be the prime focus of future studies emanating from this work, although all 16 families will be considered for further analysis.

Future studies will involve quantitative wet chemical analysis to confirm the NIR results on the outlier individuals. Those individuals for which altered cell wall chemistry is confirmed will then be subjected to fermentation analysis to determine whether or not biomass conversion process kinetics, yields and/or economics are significantly affected. In the quest to develop a commercially viable biomass conversion process, every penny saved in the production of ethanol puts us closer to the goal of a plentiful supply of an

economically competitive gasoline substitute. Those mutants that provide indications for a decrease in process cost will be pursued further to identify the gene(s) responsible for the observed changes in cell wall composition and associated changes in process economics. These genes will eventually be incorporated into maize breeding programs directed at the development of a truly dual use crop.

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References

Barrière, Y., Argillier, O. (1994). "Brown-midrib genes of maize: a review." Agronomie. 13, pp. 865-876.

Meglen, Robert R. (1992). "Examining large databases: a chemometric approach using principal component analysis." Marine Chemistry. 39, pp. 217-237.

Figure 1

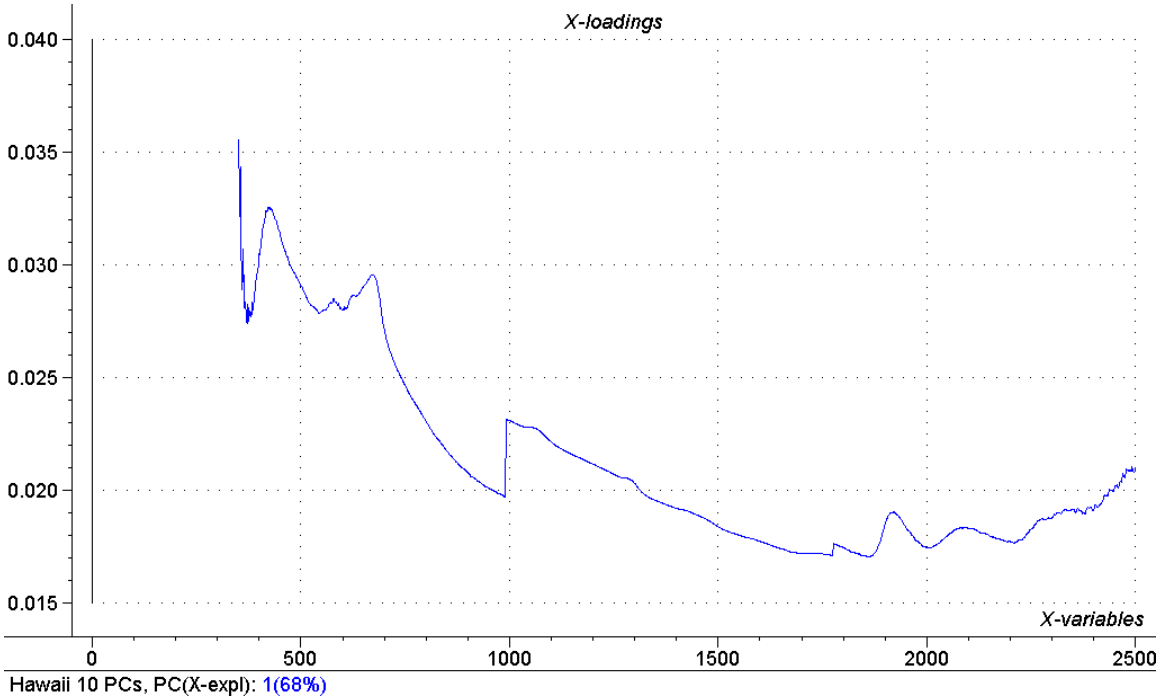
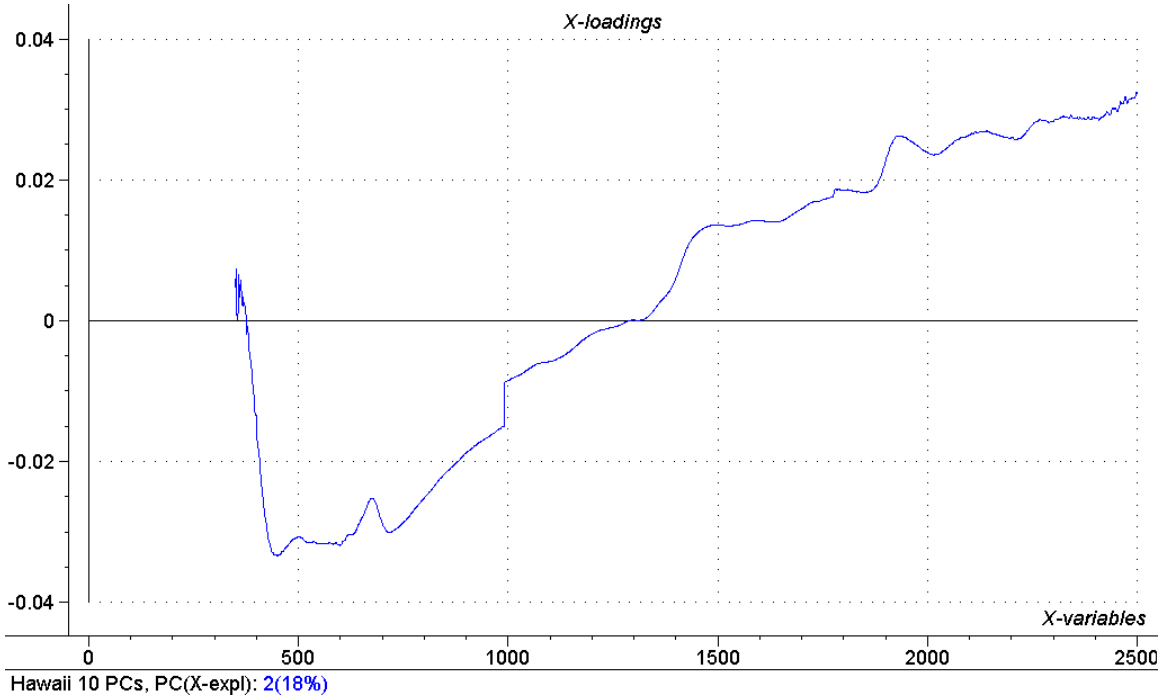


Figure 2



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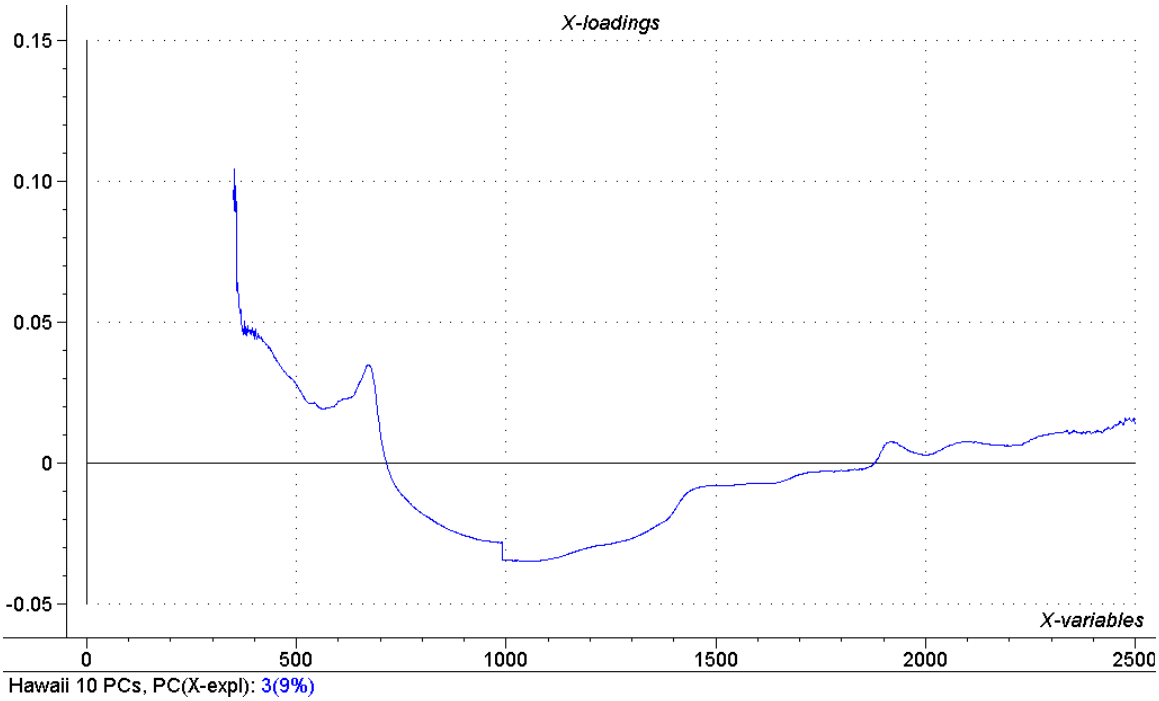

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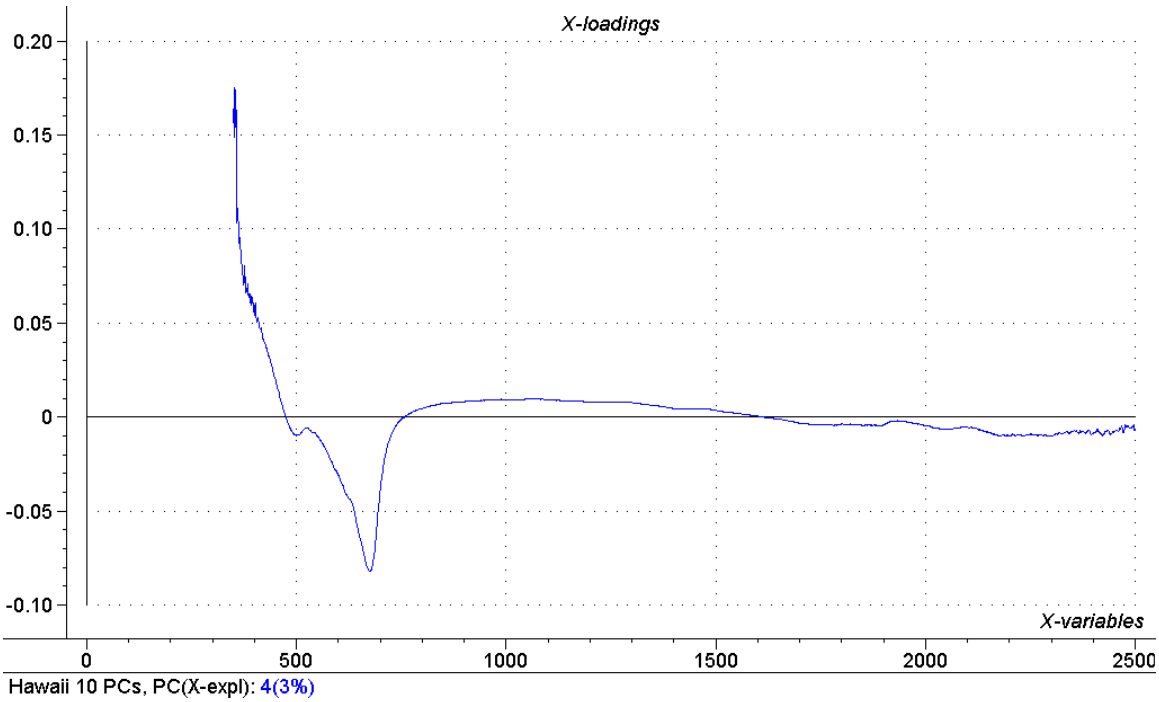

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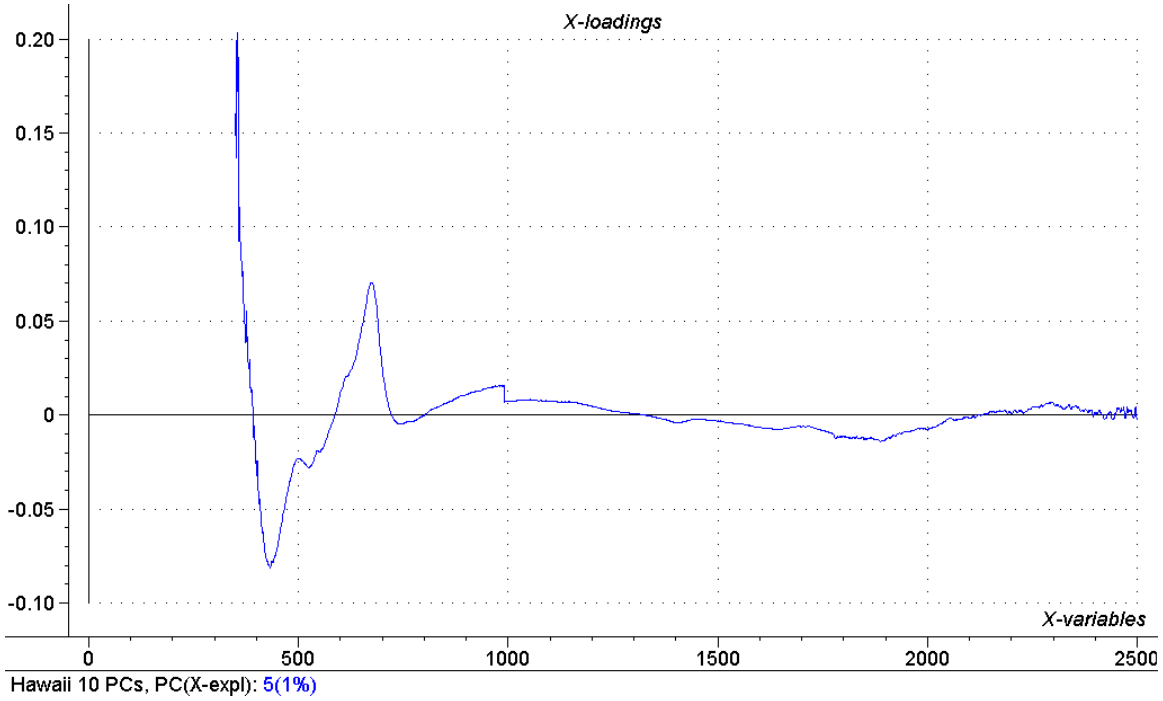

Figure 6

Figure 7

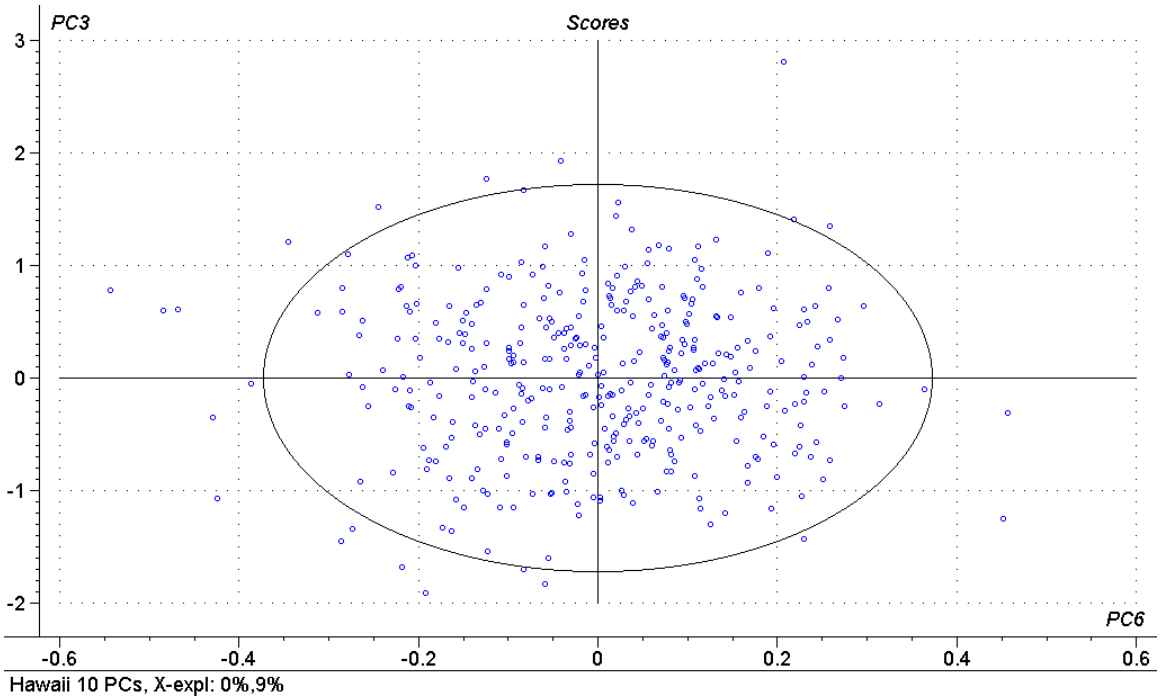

Table 1

Hawaii Family	Individual	CSHL Family	Spectrometer File Number
606	10	3668	H2.169
609	10	3671	H2.168
623	9	3961	H2.042
623	9	3961	H2.003
624	**1**	**3962**	**H2.123**
624	**3**	**3962**	**H1.013**
629	2	3967	H2.072
633	4	3971	H1.138
633	4	3971	H1.110
633	4	3971	H1.126
634	5	3972	H1.176
750	3	9170	H1.116
755	5	9176	H2.099
756	1	9177	H2.110
758	**4**	**9179**	**H1.124**
758	**8**	**9179**	**H1.141**
759	3	9180	H2.089
761	1	9602	H2.158
762	**6**	**9603**	**H2.154**
762	**7**	**9603**	**H1.002**
764	5	9606	H2.164
768	6	9610	H2.066