RESEARCH

AFLP Discrimination of Native North American and Cultivated Hop

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ABSTRACT

Hop (*Humulus lupulus* L.) is cultivated for the female flowers, or "cones," which traditionally have been used as a bittering and flavoring agent in beer. Hop breeding historically relied on relatively simple selection techniques within established breeding lines. Supplementing current breeding material with new genetic sources would enhance a hop breeder's ability to select for new traits. The objective of this research was to assess the genetic relationship of *H. lupulus* var. *lupuloides* and *H. lupulus* var. *pubescens* accessions with hop germplasm currently utilized by hop breeding programs. A total of 60 hop accessions representing both cultivated (n = 21) and native (n = 39) *Humulus* were evaluated using amplified fragment length polymorphism (AFLP) molecular markers. The four primer combinations used generated 296 scorable fragments of which 176 (59.5%) were polymorphic. Principal components analysis and hierarchal cluster analysis showed the native American accessions clustering separately from the cultivated germplasm. Within each of the two main groups, two smaller subgroups were evident with *H. lupulus* var. *lupuloides* and *H. lupulus* var. *pubescens* segregated into unique clusters. This research provides the first molecular genetic evidence of *H. lupulus* var. *pubescens* and *H. lupulus* var. *lupuloides* being two separate botanical varieties. Results from this research suggest incorporating the native American hop accessions studied in this experiment would enhance the genetic diversity within hop breeding programs.

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Abbreviations: AFLP, amplified fragment length polymorphism.

The cultivated hop (*Humulus lupulus* L.) plant is a perennial, dioecious, climbing vine and is one of two genera in the Cannabaceae family (the other being *Cannabis*). Hops have a variety of uses, the most familiar being the use of mature female flowers, or "cones," as a flavoring, bittering, and preservative agent in beer brewing. Hop vines have long fibers that are used in a similar manner to hemp (*Cannabis sativa* L.), and hops have potential uses as pharmaceuticals (Milligan et al., 1999, 2002; Stevens and Page, 2004; Zanoli and Zavatti, 2008) and as a replacement for antibiotics in livestock feed (Cornelison et al., 2006).

Early hop cultivars were likely selections from indigenous genotypes that were adapted to particular growing regions. Later, selections were made from open pollinated crosses, segregants from clonal propagation (i.e., mutations), and from superior progeny developed via hybridization (Henning, 2006; Neve, 1991). Hop improvement has focused on desirable agronomic traits, enhanced disease and pest resistance, and modification of chemical constituents for beer brewing. While agronomic traits and disease resistance are both important characteristics for breeders to pursue, brewing quality is perhaps the most difficult challenge facing hop breeders, and ultimately the trait that decides the fate of a promising genotype. The acceptance of a hop cultivar by brewmasters depends on the types and amounts of the various chemical components in hop resin. Resin glands located at the

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base of hop cone bracteoles produce a complex mixture of chemical components. The principal chemical components desired for beer brewing are the α- and β-acids for imparting bitterness and the essential oils for providing flavor and aroma. The essential oils alone are comprised of more than 200 chemical components (Neve, 1991).

Given the complexity of the hop chemical profile and the brewer's desire to produce a consistent product, the development and acceptance of a new hop cultivar, particularly aroma types, is a slow process. Hop cultivars may persist in a commercial environment for many years before disease or changing market conditions force the acceptance of a replacement cultivar. Development of a replacement hop cultivar is usually accomplished by using select female cultivars that are favored by brewers. Male genotypes often are related to, or selected from, the commonly used female cultivars. Thus, it is important for a hop breeder to assess genetic diversity within the breeding program, and new material entering the program, so that appropriate crosses are made that minimize inbreeding.

Cultivated hop is derived from *H. lupulus* which has five recognized botanical varieties: *H. lupulus* var. *lupulus,* endemic to Europe and introduced in eastern North America; *H. lupulus* var. *pubescens*, found in the midwestern United States; *H. lupulus* var. *lupuloides*, indigenous to central and eastern North America; *H. lupulus* var. *neomexicanus,* ranges from the southwestern United States to southwestern Canada; and *H. lupulus* var. *cordifolius*, found in eastern Asia, notably Japan (Small, 1978). The prevalent genetic source in modern hop cultivars, based on morphological characteristics, is *H. lupulus* var. *lupulus* with some amount of *H. lupulus* var. *lupuloides* and *H. lupulus* var. *cordifolius* having been introgressed as well. Very little *H. lupulus* var. *pubescens* has been detected in modern cultivars, which suggests this might be a source for new genes (Hampton et al., 2001; Small, 1980). Native American hop genotypes are potential gene sources for early maturity and other agronomic characteristics, disease and pest resistance, higher α- and β-acid potential, and chemical compounds with potential pharmaceutical uses (Hampton et al., 2001; Haunold et al., 1993; Smith, 2005; Stevens et al., 2000).

Assessing the genetic diversity within a hop breeding program traditionally was accomplished with pedigree records. If pedigree records are not available, molecular tools can play an important role in ascertaining the relatedness of genotypes within, and entering, the breeding program. Several research groups have demonstrated the ability of various molecular marker systems to measure genetic distance among hop genotypes. Generally, genetic marker-based assessment suggests modern hop cultivars derive from European or European × native American germplasm pools (Jakse et al., 2001; Patzak, 2002; Pillay and Kenny, 1996; Seefelder et al., 2000; Townsend and Henning, 2005). In a microsatellite analysis of 124 hop accessions from Europe, Asia, and North America, Jakse et al. (2004) found that European cultivated and native hop accessions contained limited allelic variation compared to North American cultivars and native accessions. They noted that European native hops collected in the Caucasus region were genetically distinct from the rest of the European hops studied. More recently, Murakami et al. (2006b) corroborated these results by analyzing both nuclear and chloroplast DNA. Stajner et al. (2008) divided native European hop accessions into three genetic groups using 29 microsatellite markers. Although a few native American accessions have been characterized by molecular markers, previous research has reported native American accessions by collection location, not botanical variety. Given that the three indigenous native American varieties overlap in geographic distribution (Small, 1978), it is extremely difficult to determine which botanical varieties were analyzed.

An important aspect for incorporating new genetic material into a breeding program is ascertaining its genetic diversity. The objective of this research was to characterize the genetic relationship of *H. lupulus* var. *lupuloides* and *H. lupulus* var. *pubescens* accessions with hop cultivars utilized by hop breeding programs.

MATERIALS AND METHODS

Plant Materials

Sixty hop accessions were chosen for evaluation representing a broad range of cultivated and native-type genetic diversity. Native *Humulus* accessions studied were clonally propagated from six *H. lupulus* var. *pubescens* genotypes collected in Missouri and 33 *H. lupulus* var. *lupuloides* genotypes collected at locations in North Dakota and in Manitoba and Saskatchewan, Canada (Table 1). These accessions are part of the USDA-ARS hop breeding and genetics program and include both female and male genotypes. Plants were grown in a field nursery near Corvallis, OR, and managed in the same manner as a typical commercial hop yard in the U.S. Pacific Northwest.

Amplified Fragment Length Polymorphism (AFLP) Analysis

Young leaf tissue was harvested, rinsed with deionized water, blotted dry, and stored at –80°C before analysis. Frozen tissue was lyopholized at –40°C for 24 h, then at –20°C for another 24 h, and stored at –20°C before DNA extraction. Nucleic acids were extracted from 20 mg of lyopholized tissue using the DNeasy Plant Mini Kit (Qiagen, Inc, Valencia, CA). The AFLP protocol used was previously described for hops by Townsend et al. (2000). Primer sequences used are listed in Table 2. Selective amplification was performed with primer combinations eAGC-mCAG, eACC-mCAC, eAAC-mCAG, and eAAC-mCTC. Gel bands were visualized on an ABI 3100 DNA sequencer (Applied Biosystems, Foster City, CA).

Data Analysis

Gel images were scored and a binary data matrix was created using Genographer software (Benham et al., 1999). All data analysis was

Table 1. Collection locations and pedigree information for 60 *Humulus* accessions evaluated by amplified fragment length polymorphism (AFLP) molecular markers.

Genotype	Pedigree/identifier	Origin	Type†	Cluster‡
1019-4-33	*Humulus lupulus var. pubescens; 'Rulo-E'*	Missouri, USA	N	A1-1
1019-5-01	*H. lupulus var. pubescens; 'Rulo-E'*	Missouri, USA	N	A1-1
1019-5-03	*H. lupulus var. pubescens; 'Rulo-E'*	Missouri, USA	N	A1-1
1019-5-04	*H. lupulus var. pubescens; 'Rulo-E'*	Missouri, USA	N	A1-1
1019-5-05	*H. lupulus var. pubescens; 'Rulo-E'*	Missouri, USA	N	A1-1
1019-1-27	*H. lupulus var. pubescens; 'Rulo-E'*	Missouri, USA	N	A1-1
1000-47F	*H. lupulus var. lupuloides; 'Souris-E2'*	Manitoba, Canada	N	A1-2
1000-13M	*H. lupulus var. lupuloides; 'Souris-E2'*	Manitoba, Canada	N	A1-2
1003-04F	*H. lupulus var. lupuloides; 'Burlington-N #2'*	North Dakota, USA	N	A1-2
1003-11M	*H. lupulus var. lupuloides; 'Burlington-N #2'*	North Dakota, USA	N	A1-2
1003-18M	*H. lupulus var. lupuloides; 'Burlington-N #2'*	North Dakota, USA	N	A1-2
1004-04	*H. lupulus var. lupuloides; 'Minot-E'*	North Dakota, USA	N	A1-2
1004-05	*H. lupulus var. lupuloides; 'Minot-E'*	North Dakota, USA	N	A1-2
1005-02	*H. lupulus var. lupuloides; 'White Earth-S'*	North Dakota, USA	N	A1-2
1005-05M	*H. lupulus var. lupuloides; 'White Earth-S'*	North Dakota, USA	N	A1-2
1005-12F	*H. lupulus var. lupuloides; 'White Earth-S'*	North Dakota, USA	N	A1-2
1005-14M	*H. lupulus var. lupuloides; 'White Earth-S'*	North Dakota, USA	N	A1-2
1015-01M	*H. lupulus var. lupuloides; '4 Qu'Appelle'*	Saskatchewan, Canada	N	A1-2
1001-03F	*H. lupulus var. lupuloides; 'Logan-N'*	North Dakota, USA	N	A1-2
1001-21M	*H. lupulus var. lupuloides; 'Logan-N'*	North Dakota, USA	N	A1-2
1002-04F	*H. lupulus var. lupuloides; 'Burlington-N'*	North Dakota, USA	N	A1-2
1002-18F	*H. lupulus var. lupuloides; 'Burlington-N'*	North Dakota, USA	N	A1-2
1002-26F	*H. lupulus var. lupuloides; 'Burlington-N'*	North Dakota, USA	N	A1-2
1002-27M	*H. lupulus var. lupuloides; 'Burlington-N'*	North Dakota, USA	N	A1-2
1006-16F	*H. lupulus var. lupuloides; 'White Earth-S2'*	North Dakota, USA	N	A1-2
1006-20M	*H. lupulus var. lupuloides; 'White Earth-S2'*	North Dakota, USA	N	A1-2
1006-22F	*H. lupulus var. lupuloides; 'White Earth-S2'*	North Dakota, USA	N	A1-2
1007-02	*H. lupulus var. lupuloides; 'Little Knife-E'*	North Dakota, USA	N	A1-2
1008-04	*H. lupulus var. lupuloides; 'Oxbow-S'*	North Dakota, USA	N	A1-2
1008-05	*H. lupulus var. lupuloides; 'Oxbow-S'*	North Dakota, USA	N	A1-2
1008-20	*H. lupulus var. lupuloides; 'Oxbow-S'*	North Dakota, USA	N	A1-2
1009-04	*H. lupulus var. lupuloides; Indian Head-N'*	Saskatchewan, Canada	N	A1-2
1010-01F	*H. lupulus var. lupuloides; 'Bridge 2-S'*	Saskatchewan, Canada	N	A1-2
1010-02	*H. lupulus var. lupuloides; 'Bridge 2-S'*	Saskatchewan, Canada	N	A1-2
1010-15M	*H. lupulus var. lupuloides; 'Bridge 2-S'*	Saskatchewan, Canada	N	A1-2
1010-16F	*H. lupulus var. lupuloides; 'Bridge 2-S'*	Saskatchewan, Canada	N	A1-2
1011-09F	*H. lupulus var. lupuloides; '2 Qu'Appelle'*	Saskatchewan, Canada	N	A1-2
1012-08M	*H. lupulus var. lupuloides; '3 Qu'Appelle'*	Saskatchewan, Canada	N	A1-2
1018-09F	*H. lupulus var. lupuloides; 'Fort Ransom'*	North Dakota, USA	N	A1-2
Hallertauer Mittelfrüh (21014F)	German landrace	Germany	C	A2-1
Fuggle N (21016F)	Clonal selection from Fuggle	England	C	A2-1
Cascade (21092F)	Fuggle//Serebrianca/Fuggle-s§/3/open pollinated	USA	C	A2-2
Wye Target (21112F)	Northern Brewer/Wye 22–56//Eastwell Golding/OB79	England	C	A2-2
Tardif de Bourgogne (21169F)	France landrace	France	C	A2-2
Nugget (21193F)	Brewer's Gold//Early Green/Unknown-s/3/ Brewer's Gold//East Kent Golding/Bavarian-s	USA	C	A2-2
Perle (21227F)	Northern Brewer/German 63-5-27	Germany	C	A2-1
Northern Brewer (21093F)	Brewer's Gold/OY1//Canterbury Golding	England	C	A2-1
Wye Viking (21283F)	Svalof//Bramling Cross/Wye 1-63-42	England	C	A2-1
Saazer 36 (21521F)	Clonal selection from Saazer	Czech Republic	C	A2-1
Omega (21667F)	Wye Challenger/English male	England	C	A2-1

Table 1 Continued.

Genotype	Pedigree/identifier	Origin	Type†	Cluster‡
Magnum (21670F)	Galena/German 75-5-3	Germany	C	A2-2
Spalter Select (21674F)	German 76-18-80/German 71-16-07	Germany	C	A2-1
Orion (21675F)	Perle/German 70-10-15	Germany	C	A2-1
Kitamidori (21677F)	Japan C79-27-01/Japan C79-64-110	Japan	C	A2-2
East Kent Golding (21680F)	Old English cultivar	England	C	A2-1
Galena VF (21699F)	Meristem-tip culture from Galena	USA	C	A2-2
U.S. Tettnanger (21015F)	Unknown	USA	C	A2-1
21110M	Bullion/Zattler-s	USA	P	A2-2
21268M	Northern Brewer/4/Brewer's Gold//Early Green/Unknown-s/3/Zattler-s	USA	P	A2-1
21690M	Late Grape-s//Fuggle/Fuggle-s/3/Late Cluster-s/Fuggle-s/ 4/Late Grape-s//Fuggle/Fuggle-s/3/Late Cluster-s/Fuggle-s	USA	P	A2-2

†N, native American genotype; C, cultivar; P, male parent used in breeding.

‡Assigned cluster in Fig. 2.

§"-s" denotes seedling from genotype.

performed using the R statistical environment (R Development Core Team, 2008). Genetic distance estimates were computed using the Jaccard function in the prabclus package (Hennig and Hausdorf, 2007). A dendrogram was constructed from the genetic distance estimates using Ward's (1963) method in the agnes function of the cluster package (Maechler, 2008). Principal components analysis was performed on the binary data matrix using the princomp function in the stats package (R Development Core Team, 2008).

RESULTS

A total of 296 bands were detected by AFLP of which 176 (59.5%) were polymorphic (Table 2). Primer set eAGC-mCAG amplified the greatest number of fragments ($n = 90$) while primer set eAAC-mCAG amplified the least ($n = 59$). The most informative primer set was eAAC-mCAG which generated 64.4% polymorphic bands.

Principal components analysis of the Jaccard distance estimates suggested the genotypes could be separated into two main groups (Fig. 1). Consistent with the principal components analysis, a dendrogram showed genotypes segregating into two major groups, which were denoted A1 and A2 (Fig. 2). Group A1 contained all of the native American hop genotypes while Group A2 contained the cultivars and breeding males.

Group A1 was further divided into two subgroups, A1-1 and A1-2. Subgroup A1-1 had the smallest number of individuals and contained all of the *H. lupulus* var. *pubescens* accessions. The *H. lupulus* var. *pubescens* accessions were collected near the lower Missouri River, Missouri. Subgroup A1-2 contained the remaining native American hop genotypes, which are all *H. lupulus* var. *lupuloides* and were collected in various locations in northern North Dakota and in southern Saskatchewan and Manitoba. We were not able to further subdivide Subgroup A1-2 based on collection location.

Group A2 was subdivided into two smaller groups. Subgroup A2-1 was characterized by genotypes derived primarily from European genetic stock. These genotypes are used mainly for their aroma characteristics. Subgroup A2-2 contained individuals developed primarily by hybridization between European and native American hop parents. These genotypes are characterized by having a higher bittering acid potential and some level of powdery mildew (*Podosphaera macularis* Braun and Takamatus) resistance. In general, the subgroupings of A2 were similar to earlier hop characterization work based on genetic markers (Jakse et al., 2001; Pillay and Kenny, 1996; Seefelder et al., 2000; Townsend and Henning, 2005).

The greatest average genetic distance, on a group mean basis, was measured between the two native hop groups and the European group (A1-1 vs. A2-1, and A1-2 vs. A2-1) (Table 3). Genotypes in Subgroup A2-1 are largely devoid of native hop ancestry so this result is reasonable. The smallest average genetic distance estimates were within subgroups.

DISCUSSION

Modern hop production and utilization relies on a limited number of cultivars due to the difficulty in developing genotypes with outstanding agronomic characteristics and acceptable chemical profile for brewing, particularly for aroma hops. Most hop cultivars in use today have similar ancestry, which could pose a significant problem if new

Table 2. Summary statistics for amplified fragment length polymorphism (AFLP) analysis of 60 native and cultivated *Humulus* accessions.

Primer	Monomorphic	Polymorphic	Total	% Polymorphic
eAGC-mCAG	41	49	90	54.4
eACC-mCAC	34	47	81	58.0
eAAC-mCAG	21	38	59	64.4
eAAC-mCTC	24	42	66	63.6
Total	120	176	296	59.5
Mean ± SD	30 ± 9.2	44 ± 5.0	74 ± 14.1	

diseases or pests emerge or if new market opportunities arise. Thus it is important for plant breeders to periodically assess and introgress new germplasm sources to enhance available breeding lines so that new production challenges can be readily addressed. This research was conducted to assess the relatedness of two native American hop varieties to accessions that are a part of hop breeding programs worldwide.

Results from the cluster analysis, principal components analysis, and average genetic distance estimates calculated from the resulting groups suggested that the native hop accessions included in this study were genetically distinct from the hop cultivars used for comparison. In particular, the native American hop accessions appear to be closer genetically to hybrid cultivars (Subgroup A2-2) than to European cultivars (Subgroup A2-1). According to breeding records, very little native American hop ancestry is present in the accessions occupying Subgroup A2-1 while most of the accessions in Subgroup A2-2 have significant native hop ancestry. 'Nugget', 'Galena', and 'Magnum', all have 'Brewer's Gold' in their pedigrees, while 'Target' has contributions from both Brewer's Gold and a male seedling developed from an unknown native American genotype. The male Genotype 21110M derives from 'Bullion' (Haunold et al., 1984; Neve, 1972; Romanko et al., 1979). Brewer's Gold and Bullion are sister genotypes developed by Salmon (1934, 1938) via open pollination of the native American female BB1. The genotype BB1 was collected near Morden, MB, in 1916, and based on collection location was probably from the variety *H. lupulus* var. *lupuloides* (Hampton et al., 2002).

Genotypes from *H. lupulus* var. *pubescens* clustered together while all of the *H. lupulus* var. *lupuloides* genotypes clustered together in a separate group. The *H. lupulus* var. *lupuloides* accessions we studied were collected from North Dakota, Saskatchewan, and Manitoba. We were not able to satisfactorily subdivide the *H. lupulus* var. *lupuloides* group (A1-2, Fig. 1) into smaller subgroups based on collection location. In a recent microsatellite analysis of native hops, Murakami et al. (2006a) could not discern a clear geographical relationship with clustering patterns in European accessions even though samples were collected over a wide area. In another study, Murakami et al. (2006b) were able to separate hop accessions collected from the Caucasus region from accessions collected from the rest of Europe using both nuclear and chloroplast DNA analysis. Jakse et al. (2004) also

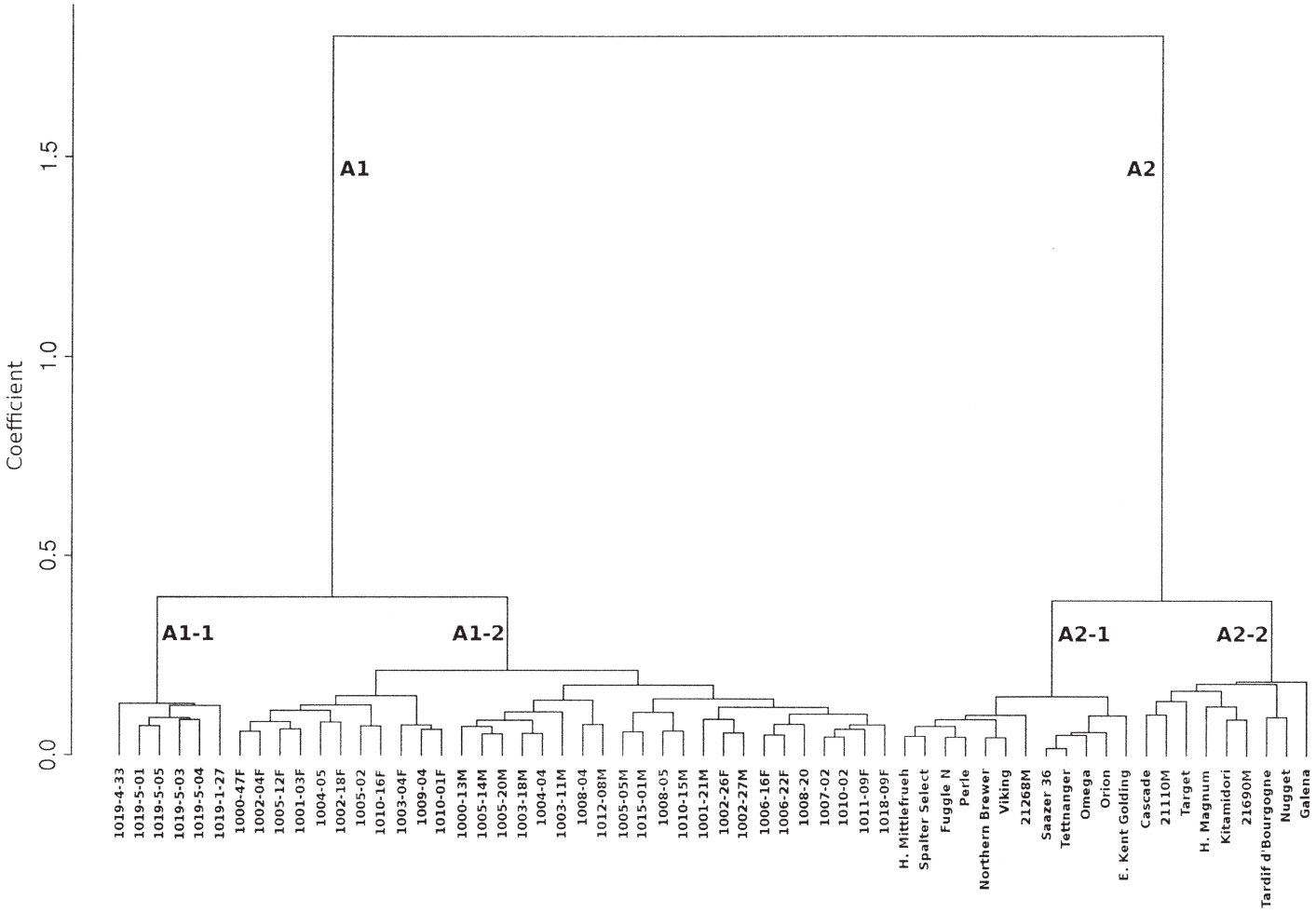

Figure 2. Dendrogram of 60 native and cultivated *Humulus lupulus* accessions computed from amplified fragment length polymorphism (AFLP) marker data.

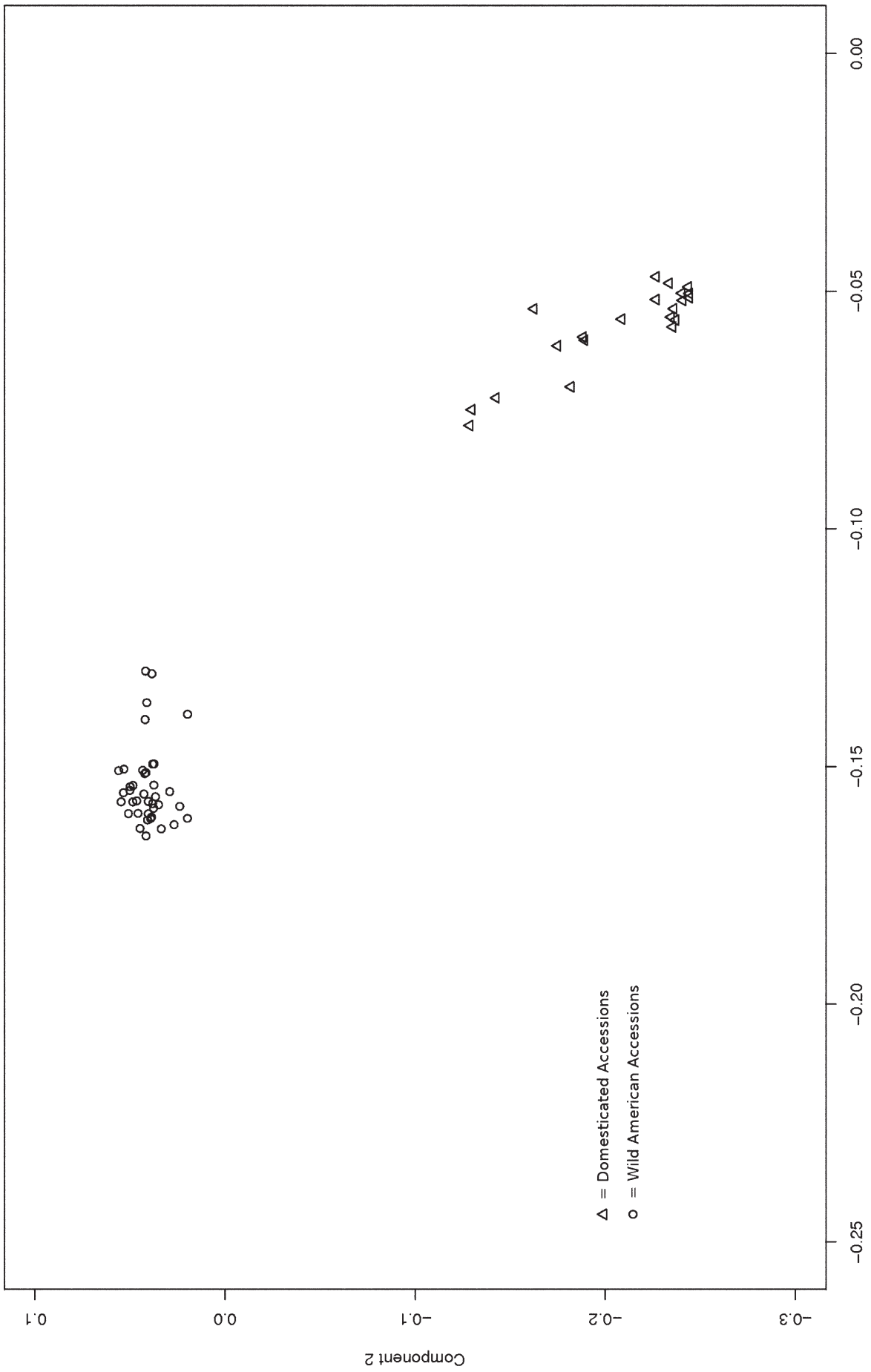

Figure 1. Principal components analysis of amplified fragment length polymorphism (AFLP) marker data from 21 cultivated and 39 native *Humulus lupulus* accessions.

found Georgian native hop accessions (Caucasus region) to differ genetically from other European hop accessions based on microsatellite assessment. Recently, Stajner et al. (2008) divided native European hops into three groups based on 29 microsatellite markers.

Our inability to subdivide *H. lupulus* var *lupuloides* accessions into smaller groups may be due to the AFLP primers that we employed for this research, the limited number of primer pairs employed (four), or perhaps a lack of genetic variation among the plants sampled. We used primer combinations that in previous research successfully resolved numerous hop breeding lines, males, and cultivars (Townsend et al., 2000; Townsend and Henning, 2005). Although these primer sets separated the two botanical varieties, and separated the cultivated genotypes from native accessions, they may not be sufficiently informative to distinguish groups within *H. lupulus* var. *lupuloides*. We had only six *H. lupulus* var. *pubescens* accessions available for this research, which is likely too small of a sample size to determine the informativeness of these primer combinations within that botanical variety. Furthermore, while four AFLP primer sets have generated significant polymorphism in previous research, even among closely related hop genotypes, a higher number of primer combinations may be needed to detect genetic patterns in native American hop accessions.

Inadequate genetic variation in the *H. lupulus* var. *lupuloides* genotypes studied is an unlikely cause for the group delineation problem within that taxon. Small (1978) classified native American hop genotypes as *H. lupulus* var. *lupuloides* if they did not neatly fit into one of the other four taxonomic varieties. This suggests these genotypes had unusual morphological characteristics, and as a group, would probably be highly diverse. This extensive diversity may require a more robust genetic analysis than provided here to ascertain relationships within *H. lupulus* var. *lupuloides*.

Table 3. *Humulus* genetic distance means, ranges, and standard deviations among and within main groups and subgroups computed from Jaccard distance estimates.

Group comparison	Mean	Low	High	Range
Tier A				
A1 vs. A1	0.114 ± 0.032	0.047	0.216	0.169
A1 vs. A2	0.366 ± 0.044	0.241	0.438	0.197
A2 vs. A2	0.130 ± 0.047	0.018	0.222	0.203
Tier B				
A1-1 vs. A1-1	0.103 ± 0.017	0.073	0.126	0.052
A1-1 vs. A1-2	0.156 ± 0.020	0.105	0.216	0.112
A1-1 vs. A2-1	0.395 ± 0.015	0.369	0.438	0.069
A1-1 vs. A2-2	0.324 ± 0.033	0.257	0.390	0.134
A1-2 vs. A1-2	0.098 ± 0.018	0.047	0.149	0.102
A1-2 vs. A2-1	0.397 ± 0.015	0.358	0.437	0.079
A1-2 vs. A2-2	0.319 ± 0.032	0.241	0.393	0.151
A2-1 vs. A2-1	0.077 ± 0.019	0.018	0.120	0.102
A2-1 vs. A2-2	0.162 ± 0.031	0.088	0.222	0.134
A2-2 vs. A2-2	0.142 ± 0.026	0.097	0.201	0.104

Incorporating new germplasm into breeding programs is important for introducing novel genes for agronomic characteristics and disease and pest resistance, and to address new market opportunities. A number of the native American hop genotypes in the present study were previously screened for powdery mildew resistance and found to be either tolerant or resistant to this devastating disease (Hampton et al., 2001; Smith, 2005). Hampton et al. (2001) also evaluated these same *H. lupulus* var. *lupuloides* accessions for growth habit, flowering habit, pest resistance, and drought tolerance. They found exceptional individuals for each of the traits evaluated, most notably resistance to the two-spotted spider mite (*Tetranychus urticae* Koch). Relatively little information is available on the agronomic characteristics, chemical attributes, and variation within *H. lupulus* var. *pubescens*. Small (1980) reported that based on morphological characters, modern hop cultivars do not appear to have *H. lupulus* var. *pubescens* as an ancestor. Small (1980) also suggested that the pubescent nature of *H. lupulus* var. *pubescens* may lend resistance against phytophagous insects.

The introduction of native germplasm into a breeding program requires the plant breeder to guard against introducing undesirable traits by carefully screening potential germplasm sources. For example, evaluating numerous native American accessions collected from 11 U.S. states and four locations in Canada, Haunold et al. (1993) observed high levels of cohumulone in most of the genotypes studied. Cohumulone may be partly responsible for an unpleasant bitterness (Rigby, 1972) and decreased foam stability in beer (Diffor et al., 1978). Furthermore, they noted that many genotypes had α-acid levels between 2 and 4%, with accompanying low alpha ratios. The alpha ratio describes the relative proportion of α-acid to β-acid in hop cones and is an indicator of bittering potential. Other undesirable characteristics that they observed include late maturity, unsatisfactory yield potential, and significant monoecy in some accessions. Evaluation of such detrimental characteristics is currently underway on the native hop accessions analyzed in this study.

A genetic survey has confirmed that numerous native American hop accessions from *H. lupulus* var. *lupuloides* and *H. lupulus* var. *pubescens* are distinct from cultivated hop accessions. This research appears to be the first genetic evidence supporting Small's (1978) classification of *H. lupulus* var. *lupuloides* and *H. lupulus* var. *pubescens* as two separate botanical varieties. These native accessions may provide novel genes for hop breeders developing cultivars for emerging markets. Additional research efforts are needed to associate desired traits to specific genetic backgrounds, and to understand inheritance patterns of these traits so that appropriate breeding strategies can be utilized for cultivar development.

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