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Title: Nuclear and Chloroplast Diversity of Pacific Northwest Wheat (*Triticum aestivum*) Breeding Germplasm .

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Wheat breeders must effectively maintain and manage existing genetic diversity in order to continue the development of superior genotypes. It is therefore fundamental that the genetic relationships and diversity within the germplasm pools be thoroughly characterized and understood. Recently, DNA-based markers have provided powerful tools for genetic diversity analysis. This study investigates the usefulness of nuclear and chloroplast simple sequence repeat (SSR) markers in characterizing Pacific Northwest wheat (*Triticum aestivum*) breeding germplasm, and explores the patterns of genetic relatedness revealed by these markers. The 15 chloroplast SSRs were effective in differentiating between B-type, D-type, and barley (*Hordeum vulgare*) cytoplasms. Genetic distance estimates were determined for each pair of lines studied and analyzed using UPGMA clustering. The markers revealed five plastomic types within the B-type cytoplasm studied. Several lines of wheat in this germplasm, including important PNW cultivars like Madsen, were found to contain D-type cytoplasm rather than the B cytoplasm of wheat. Nuclear SSR assays using 24 markers revealed three major clusters of germplasms: PNW soft white winter wheat, Western

European-derived lines, and Great Plains accessions, as well as two clusters of more distantly related lines and genetic stocks. The primary defining characteristic of these clusters was regional adaptation. Subgroups of these major groups often clustered together on the basis of pedigree and market class. When nuclear and chloroplast SSR data was combined in analysis, the primary defining characteristic of the dendrogram became the type of cytoplasm rather than regional adaptation, with secondary divisions based on pedigree relationships. Cultivars released prior to 1950 were found to have a minimum of 20% of alleles in common for nuclear and chloroplast data combined, despite being unrelated via pedigree information. Heterogeneity was 2.3% for all marker/variety combinations. Overall, these sets of markers were found to be effective in characterizing the genetic relatedness of PNW wheat breeding germplasm.

Nuclear and Chloroplast Diversity of Pacific Northwest Wheat (*Triticum aestivum*) Breeding Germplasm.

by
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I understand that my thesis will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my thesis to any reader upon request.

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Melanie Love Edwards, Author

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Introduction

The objectives of any wheat improvement program are to develop cultivars that are superior and stable in yield, resistant to biotic or abiotic stresses, and possess quality characteristic of value to the milling and baking industry. To accomplish this, wheat breeders capitalize upon the genetic diversity existing among elite germplasms, and unadapted germplasm from secondary gene pools. This genetic diversity must be properly managed to sustain long-term genetic gain. While there exist several methods for characterizing the genetic diversity based on phenotypic characteristics, comparison of the actual DNA sequence variation would provide a more direct measurement of genetic relatedness and variation. This may be accomplished through the use of DNA-based markers.

There are several DNA-based markers available and each has been used in evaluating genetic diversity of wheat germplasm. As these technologies are still under development, their applicability in wheat breeding germplasm remains to be fully explored. The Pacific Northwest wheat germplasm base is very appropriate to evaluating marker technologies. The germplasm of the Pacific Northwest includes cultivars from lines from around the world and spanning many different market

classes. Understanding of genetic relatedness of these lines could assist wheat breeders in management of diversity and developing long-term strategies to better exploit that diversity.

The objectives of this study were fourfold: i) to investigate the usefulness of simple sequence repeat markers for characterizing Pacific Northwest wheat breeding germplasm, ii) to analyze the cytoplasmic diversity available in this germplasm pool, iii) to explore nuclear diversity of this germplasm and to determine biological significance of genetic relatedness based on nuclear SSR markers, and iv) to determine the usefulness of a combined nuclear/chloroplast SSR data set and to investigate heterogeneity and underlying historical relatedness with this combined data set.

Literature Review

Maintenance of Genetic Variation

Crop improvement is accomplished by exploiting genetic diversity through breeding and selection. Plant breeders create diversity and maximize genetic gain through recombination of allelic variation. This is accomplished through various methods of crossing, backcrossing, selfing, cytoplasmic manipulation, and recurrent selection (Poehlman and Sleper 1995). Plant breeders use an array of strategies, from wide crosses with alien species to crosses among elite inbred lines, in attempts to maximize genetic gain to meet both long and short-term goals. Allelic variation is imperative for ongoing crop improvement efforts, and thus it is an important goal for breeders to effectively maintain and manage genetic diversity within their breeding germplasm.

Within a particular breeding program, the germplasm base can contain a wide variety of genetic material, from elite advanced lines to wild relatives or varieties from other regions. Germplasm pools also include breeding lines with specific alleles of interest, such as those for disease resistance, which may be deficient in many other traits. Prior to the initiation of genetic diversity studies, allelic variation existing within a breeding germplasm base was unclear. Assumptions regarding genetic diversity and similarity were based on pedigrees and phenotypic evaluation of important traits. Statistical

means of quantifying genetic variation were developed using pedigree analysis (Kempthorne 1969) or phenotypic data (Jain et al. 1975).

In the 1970's, concern arose over lack of diversity, or uniformity, among commercial varieties developed through modern plant breeding. The United States experienced an epidemic of southern corn (*Zea mays* L.) leaf blight, caused by the fungus *Bipolaris maydis*, which destroyed 15% of the nation's crop in 1970-71. At that time, 80% of the hybrid corn in the U.S. was planted with corn produced using male sterile Texas cytoplasm. Although this cytoplasm was useful for efficient production of hybrid seed, it rendered the crop vulnerable to southern corn leaf blight. The epidemic and crop losses led the 1972 US National Academy of Sciences to conduct an assessment of genetic vulnerability of major crops. The Council was concerned that modern breeding practices were systematically reducing genetic variability and biodiversity. Of particular concern to the Council was the use of narrow elite germplasm pools the focus of many breeding programs. Given a limited pool of elite, highly related germplasm, alleles have the potential to become rapidly fixed, through either natural or artificial selection, thus reducing the diversity of the germplasm pool and resulting cultivars.

The loss of alleles with neutral or adverse effect on agronomic traits may not reduce the potential for future crop improvement. However, given the complex nature of the genetic background, including epistasis, heterosis, and possible epigenetic effects, the

long-term consequences of the loss of alleles is unpredictable and could be highly negative. Therefore, researchers found the need to more effectively study, monitor, and manage genetic variation of germplasm pools and related species.

Rasmusson and Phillips (1997) suggested that new genetic variation can be generated without the addition of new genetic stocks. Using a study of elite germplasm from barley (*Hordeum vulgare L.*), they showed that selection gain continues even when using a narrow pool of elite lines. As these lines were presumed to be fixed for many of the same positive alleles, the authors argue that this gain may be due to *de novo* variation, epistasis, and/ or epigenetic effects. The variation might arise from well-characterized genetic processes such as mutation, unequal crossing over, intragenic recombination, transposable element activity, DNA methylation, paramutation, or gene amplification. New alleles produced through these processes can have substantial effects on the plant phenotype due to epistasis, the interaction of a gene with a new genetic environment. Others reject their hypothesis (Smale et al. 2001), but there is little evidence to refute their premise. Evidence for the existence of *de novo* variation could dramatically alter strategies for long-term breeding and management of diversity. Whether genetic diversity is generated *de novo* or whether it is incorporated from existing allelic variation, quantification of available diversity remains an important goal in order to efficiently manage diversity and ensure continued genetic gain.

Quantification of Diversity

Prior to the availability of molecular markers, genetic diversity estimates were based on measures of coefficient of parentage (COP) and relatedness based on pedigree (Kempthorne 1969). While these measurements provide an estimate of relatedness among germplasm, the estimates are based on several false assumptions. Similarity estimates based on coefficients of parentage assume the following: i) parental lines of unknown pedigree are unrelated, ii) there is no selection pressure, and iii) both parents contribute equally in a cross. Later studies have shown that parental lines previously presumed to be completely unrelated actually may have up to 30% genetic similarity (Kim and Ward 1997, Melchinger et al. 1994, Russel et al. 1997), which undermines the first assumption of COP. The second assumption is often violated because breeding programs apply selection pressure for agronomically important attributes. Natural selection also exerts pressure on populations of crop plants. Finally, studies using molecular markers have confirmed that parents often do not contribute equally to the genetic material of their offspring (Lorenzen et al. 1995, Paull et al. 1998). Thus, pedigree comparisons are generally recognized as an ineffective means to quantify genetic diversity.

Elegant statistical approaches have been developed to quantify genetic variation in structured populations (Dudley 1982, Lynch and Walsh 1998). However, as breeding germplasm may not exist in the appropriate structured populations, this method is not applicable when analyzing diversity of a broad germplasm base. Morphological

markers were found to be useful in cases with high levels of diversity in important characters (Donini et al. 2000). Application is limited, however, by the available number of segregating morphological traits. Other studies have evaluated gene frequencies using electrophoretic patterns of isozymes or disease-resistance loci (Cox et al. 1986, Eagles et al. 2001, Sharp et al. 2001). However, the methods require knowledge and measurement of specific genes and do not address overall diversity. With the development of molecular marker technologies, DNA-based markers provide new, powerful, and efficient tools for measuring genetic diversity. Genetic diversity studies to date have used markers based on restriction fragment length polymorphism (RFLPs), amplified fragment length polymorphism (AFLPs), random amplified polymorphic DNA (RAPDs), simple sequence repeats (SSRs) or microsatellites, and inter simple sequence repeats (ISSRs). Each of these markers provides a measurement of genetic similarity or dissimilarity by comparing differences at the DNA sequence level. While a comparison of actual DNA sequences would provide a more complete measure of genetic diversity, limitations on resources make this impractical for full genome comparison.

DNA-based Markers

The most frequently used type of molecular marker to date has been RFLPs, due to their high level of distinguishable polymorphism and the ability to generate probes without prior sequence knowledge. RFLPs are detected by the use of restriction enzymes that cut genomic DNA at specific sequences, resulting in DNA fragments of

different sizes. RFLPs are visualized using Southern blot analysis with labeled probes that are either random genomic DNA or copies of transcribed genes (cDNA). RFLPs are normally codominant, as heterozygotes and homozygotes can be distinguished. Although there are few alleles per locus, RFLPs are useful as they are abundant throughout the genome (Lynch and Walsh 1998). They do, however, require a large amount of purified DNA for analysis (15 to 20 ug for wheat). The use of radioactivity also makes them a less desirable marker for high throughput diversity analysis.

PCR-based markers are becoming more frequently used, as these do not require radioactivity and the products are often relatively simple to visualize and score. They have been developed using three different strategies (Staub et al. 1996): i) markers that are amplified using single random primers, such as RAPDs, ii) markers using selective amplification of endonuclease-digested DNA fragments, i.e. AFLPs, and iii) markers using two primers that measure the variability of the target sequence between them (ISSRs and SSRs).

Short, randomly amplified pieces of DNA are the basis of markers in RAPDs. They are highly abundant within the genome. They offer no information about the location of the DNA being amplified, but provide opportunities for fingerprinting DNA about which little is known. They are dominant, in the sense that they do not allow for distinction between homozygous or heterozygous states. They are codominant in some cases, as occasionally RAPD bands of differing lengths can be assigned to the same

locus. They are visualized using ethidium bromide-stained agarose gels. They allow for the opportunity to amplify several different loci using a single primer, and require a minimal amount of DNA for analysis (10-25 ng). On the other hand, they are generally considered unreliable due to a lack of repeatability in the fingerprints that are generated, and due to the random nature of the primers used.

AFLPs are dominant, multilocus markers that exhibit high levels of polymorphism, and offer high resolution. They are produced using DNA markers of random origin on DNA fragments resulting from the action of restriction enzymes (Vos et al. 1995). These are visualized by using primers that are either flourescently or radioactively labeled. AFLPs are useful because many markers can be generated with minimal primer testing, are highly polymorphic, and are relatively easy to score. Yet, these polymorphisms are less facile in interpretation. The multilocus nature of these markers reduces the ability to compare different alleles of the same locus to one another, since all alleles are measured on a presence/absence basis, regardless of locus.

SSRs are codominant loci that are highly polymorphic. SSRs have an added advantage in that the chromosomal locations for these are frequently known. Two primers are used in generating SSRs that are complementary to sequences on either side of a series of simple repeats. These repeats (di, tri, or tetranucleotide) differ in length, and these polymorphisms can be visualized using fluorescent labeling and electrophoresis on either agarose or polyacrylamide gels. The primers are developed from known

sequences. As more sequence data becomes available from large-scale genome projects, the number of species-specific SSRs is also increasing. Dinucleotide repeats are most commonly used. However, due to scoring difficulties, some researchers are choosing to employ more trinucleotide SSRs (M. Warburton, personal communication).

ISSRs are another PCR based marker technology that uses simple sequence repeats as the primer and measures the polymorphism of regions between these repeats. Nagaoka and Ogihara (1997) showed that ISSR polymorphism is similar to that of RFLPs in wheat, and higher than that obtained from RAPDs. As with SSRs, some of these markers are mapped, yet precise sequence information is unnecessary to produce them. Several studies have compared the efficacy of each of these molecular markers. Choice of marker technology depends largely on the objectives of each individual study, the time and financial resources available, equipment, qualification requirements, safety concerns, and availability of markers.

Wheat

Breeding efforts on wheat have been underway since the early 1800s. These efforts have impacted wheat's architecture, yield, grain quality, and biotic and abiotic stress resistance, and presumably, genetic diversity within this species. Wheat (*Triticum aestivum* L.) often is considered "the most valuable single crop in the modern world" (Diamond 1997) and has been the subject of intense improvement efforts. It has been

cultivated in southwestern Asia, its geographic center of origin, for more than 10,000 years. It is predominantly a self-pollinated species. Individual cultivars, lines, and plants tend to be homozygous as alleles have become fixed through self-pollination.

Wheat is an allohexaploid with a comparatively large genome (13.3 gigabases), approximately three times larger than the genome of humans or corn. Modern bread wheat is the result of ancient hybridization events involving three different, related ancestral species. The three different genomes (A, B, and D) found in wheat are mostly homoeologous (orthologous) to one another and often contain duplications of genetic material. The large chromosome number (2n=6x=42) of wheat and polyploidy have been an obstacle to studying the genetics of wheat. However, its significance to human society and its long history of breeding make it an important choice for study of genetic diversity. Additionally, over 400,000 accessions are housed in collections all over the world, allowing for large-scale studies of *a priori* genetic diversity and providing a broad genetic base for future breeding efforts (Poehlman and Sleper 1995).

In the past century, major advances in yield and productivity have been achieved in wheat. In the 1950's, a concerted international breeding effort began that when combined with intense management, ultimately led to the "Green Revolution," which changed the management practices for wheat production (Poehlman and Sleper 1995). New semidwarf varieties of wheat were developed and released by the International

Maize and Wheat Improvement Center (CIMMYT) that were able to grow under high fertilization without lodging, significantly increasing yield. These varieties were distributed worldwide through cooperative release and breeding efforts. In some cases, these new wheat varieties have replaced local landraces. The rapid adoption of semidwarfs and loss of local landraces increased concerns over genetic variability of crops and interests in quantifying allelic variation in germplasm.

Wheat Diversity

In response to recent concerns over narrowing genetic pools, and impact from the "Green Revolution", CIMMYT undertook a diversity study of all "CIMMYT-related" bread wheat from 1965 to 2000 (Smale et al. 2001). This study examined diversity at a molecular level using genetic markers. It also examined potential loss of variation due to the declining presence of landraces in the germplasm pools. The authors used several types of molecular markers as well as pedigree information to study the diversity remaining after intense breeding. They rejected the hypothesis of genetic narrowing in modern plant breeding efforts based on i) the reports regarding the selection of parental lines and ii) evidence of high allelic variation as determined using DNA-based markers. Although there are a limited number of *major* parental lines used in modern breeding programs, these programs incorporated a large number of landraces as donors of alleles for desirable traits (Smale et al. 2001). While these donors do not provide the genetic backbone of the released varieties, their utilization

refutes the assertion that breeding programs discard available diversity and utilize only elite cultivars for crossing.

Smale et al.'s study (2001) further addressed changes in diversity across time. While diversity estimates for each decade did vary, there was no linear correlation, and no significant difference among cultivars in the earliest decade studied versus among those in the most recent decade. The essence of these findings is similar to a study by Manifesto et al. (2001), where no evidence was found for the loss of genetic variation in Argentinean spring-wheat cultivars released from 1932 to 1995. While the amount of diversity did fluctuate over decades, no significant trend was found. As with the study by Smale et al. (2002), Manifesto et al. (2001) concluded that the genetic diversity had indeed changed qualitatively, but not quantitatively. A study of wheat from the United Kingdom since the 1930's which used AFLPs, SSRs, storage proteins, and morphological characteristics likewise found no indication of decrease in diversity over time (Donini et al. 2000).

Selection of Markers for Wheat Diversity Studies

Studies of diversity in wheat and its closest relatives have used RFLPs (Kim and Ward 1997, Miyashita et al 1994, Siedler et al. 1994), AFLPs (Barrett and Kidwell 1999, Warburton et al. 2002), RAPDs (Joshi and Nguyen 1993, Stoutjedijk et al. 2001), SSRs (Huang et al. 2002, Ishii et al. 2001, Plaschke et al. 1995, Prasad et al 2000,

Roeder et al. 1998), ISSRs (Nagaoka and Ogihara 1997) and combinations of the above (Donini et al. 2000, Souza et al. 1994).

As previously noted, RFLPs are not a desirable marker for wheat due to the low level of polymorphism. While useful when other markers are unavailable, RAPDs are considered inferior because they are not repeatable and their chromosomal location is not normally known.

Barrett and Kidwell (1998) advocated the use of AFLPs rather than SSRs for wheat diversity studies, as the mean number of polymorphic bands per SSR primer is less than half that detected by AFLPs among the hexaploid wheats in their study. AFLP bands, however, may represent a number of loci and therefore may be difficult to score. Additionally, higher polymorphism is not necessarily indicative of a more optimal marker. In a study that used AFLPs to distinguish sister lines of Bobwhite, Warburton et al. (2002) found that the presence of wheat-alien translocations could bias genetic diversity estimates when using unmapped markers such as AFLPs.

Many researchers advocate the use of SSRs, as several hundred of these markers are mapped and publicly available (Fahima et al. 2002, Hammer et al. 2000, Pestsova et al. 2000, Plaschke et al. 1995, Roeder et al. 1998, Stephenson et al. 1998). The abundance of simple repetitive DNA in a genome has been attributed to slipped strand mispairing (Levinson and Gutman 1987). This process is believed to be a ubiquitous

force in the evolution of eukaryotic genomes. SSRs are thought to represent stepwise mutations, providing inference about phylogenetic relationships. However, it is important to point out that mutation rate in SSRs is high, and there are limited numbers of alleles per marker. Nauta and Weissing (1996) warn that many SSR loci, "much more than 15," are necessary to correctly infer a given phylogenetic relationship. A recent study has suggested that some of the variation at microsatellite loci may come from insertions or deletions (indels) in the flanking region rather than in the repeat motifs (Matsuoka et al. 2002). This reduces the ability to distinguish relationships as the stepwise mutation hypothesis becomes inaccurate. The relative contribution of indels and repeat length to the variation of SSR markers remains to be examined.

Regardless of phylogenetic limitations of microsatellites, SSRs remain useful to quantify the diversity within germplasm, as well as many other possible uses. Plaschke et al. (1995) suggested the use of SSRs for wheat cultivar identification. This is of increasing interest as intellectual property rights become more of an issue in crop improvement. Manifesto et al. (2001) found that a set of 10 SSRs were able to conclusively differentiate between 105 lines of bread wheat.

ISSRs may provide another valuable tool for cultivar identification. A study using a series of 33 polymorphic ISSRs found that each was able to distinguish among 6 lines of cultivated wheat (Nagaoka and Ogihara 1997). Whether more ISSRS will be

needed or whether this set of ISSRs can distinguish closely related lines remains to be explored.

Chloroplast SSRs

Chloroplast DNA is a circular molecule that is maternally inherited in wheat and grass species. For these reasons, the chloroplast genome can be studied as a haplotype (Ishii et al. 2001). Previous studies in soybean have shown that closed populations of inbreeding crop species can remain genetically diverse for as many as 50 generations in a given area (Allard 1988). Lee et al. (1994) used chloroplast and mitochondrial DNA RFLPs to determine the number of distinct cytoplasmic genotypes in soybean. A group of females were chosen based on diverse phenotypes for development of new populations. These were found to have few cytoplasmic types, despite the higher levels of phenotypic variation. When these females were placed in a forced outcrossing situation, the cytoplasmic diversity declined substantially through subsequent crossings. This implied that some chloroplast interactions affected the fitness of the outcrossed offspring. The full implications of chloroplast/nuclear interactions remain unclear.

Variation in chloroplast DNA in ancestral species of wheat has been studied previously using RFLPs (Miyashita et al 1994). Chloroplast haplotypes within cultivated wheat have not been thoroughly investigated. Using the DNA sequence of the wheat chloroplast genome (Ogihara et al. 2000), Ishii et al. (2001) developed 24

chloroplast SSR (cpSSRs). Twenty-one of these were found to be polymorphic, and were used to differentiate chloroplast (or plastome) haplotypes in wheat and its ancestral species. The values of diversity using these cpSSRs were found to match those determined by RFLP analysis. Although some have warned against using SSRs for phylogenetic evaluation due to size homoplasy (Doyle et al. 1998), Ishii et al. (2001) dismissed these concerns, noting that the polymorphic loci were from different regions of the genome and therefore would not be seriously affected by size homoplasy at individual loci. There was a high level of haplotype diversity between species, and some diversity was noted within species, even using a small number of lines.

Although cpSSRs have been used to differentiate closely related species, no studies have addressed plastome diversity within a cultivated species using cpSSRs. While Ishii et al.'s (2000) initial study indicates that common wheat has at least two chloroplast haplotypes, due to the high variability noted in cpSSRs, the possibility exists that there are several haplotypes that have not yet been identified. To date, the amount of chloroplast haplotype diversity within cultivated wheat has not been explored.

Materials and Methods

Plant Materials

In order to get an initial picture of diversity available in PNW wheat breeding germplasm, the genotypes included were selected to represent the major classifications that have been important in the PNW. This study included 174 genotypes chosen to represent significant parental lines of cultivars developed in the Pacific Northwest, commonly grown cultivars, experimental lines, and lines of interest that have similar adaptations of unique traits of value to Oregon State University's wheat breeding program. Appendix 1 lists all accessions used, their Plant or Collection Identification numbers, and the source of seed used for this study. Appendix 4 lists the pedigrees for several of the major lines included in this study. All plants were grown under greenhouse conditions in Corvallis, OR for approximately 3 weeks before they were harvested for DNA extraction.

DNA Extraction

DNA extraction was performed using a protocol described by Liu and Whittier (1994) with modifications to increase throughput. DNA was extracted from a bulk of leaves from eight plants of each seed source. Tissue was kept on ice throughout the extraction procedure except during centrifugation. A 30-50 mg sample of total bulked tissue was cut into 1 cm pieces and placed in a 1.5 mL vial. Then 400 uL of cold DNA isolation buffer [10 mM Tris-HCl pH 9.5, 10 mM EDTA, 100 mM KCl, 0.5 M

sucrose, 4 mM spermidine, 1.0 mM spermine, 0.1 % (v/v) 2-mercaptoethanol, 2% (w/v) sarkosyl] was added to each tube. The samples were ground by placing a tungsten carbide bead in each tube and using a mixer mill (Retsch MM 300 USA) according to the manufacturer's directions for 1 min 30 sec at 30 sec^{-1} on each side. Then, the tubes were centrifuged for 5 min at ~6000 g. After removing the tungsten beads with a magnet, 250 uL of phenol:choloroform (1:1 mixture) was added to each tube. The tubes were then mixed gently by hand and centrifuged for 25 min. at ~6000 g. The aqueous layer (300 uL) containing the DNA was transferred to new tubes. Approximately 30 uL of 3 M sodium acetate (pH 5.0) and approximately 750 uL of cold absolute ethanol were added to the supernatant to precipitate the DNA. These were mixed well and then centrifuged at ~6000 g for another 30 min. The alcohol was removed from the DNA pellet, and 0.5 mL of cold 70% ethanol was added to each tube to remove the salt. The tubes were again centrifuged for 30 min at ~6000 g. The alcohol was drawn off and pellets allowed to air-dry completely. DNA was dissolved in TE buffer (pH 8.0) containing 10 ug mL^{-1} RNase to a concentration of 50 ng/uL and stored at –20 °C until used in PCR cycling.

SSR Markers

The nuclear SSR loci used in this study (Table 2) were developed by Roeder et al. (1998), and chloroplast SSR loci in this study (Table 1) were developed by Ishii et al. (2000). The nuclear markers are distributed throughout the genome with two chromosomes left uncovered (Figure 1). Primers were synthesized by MWG Biotech

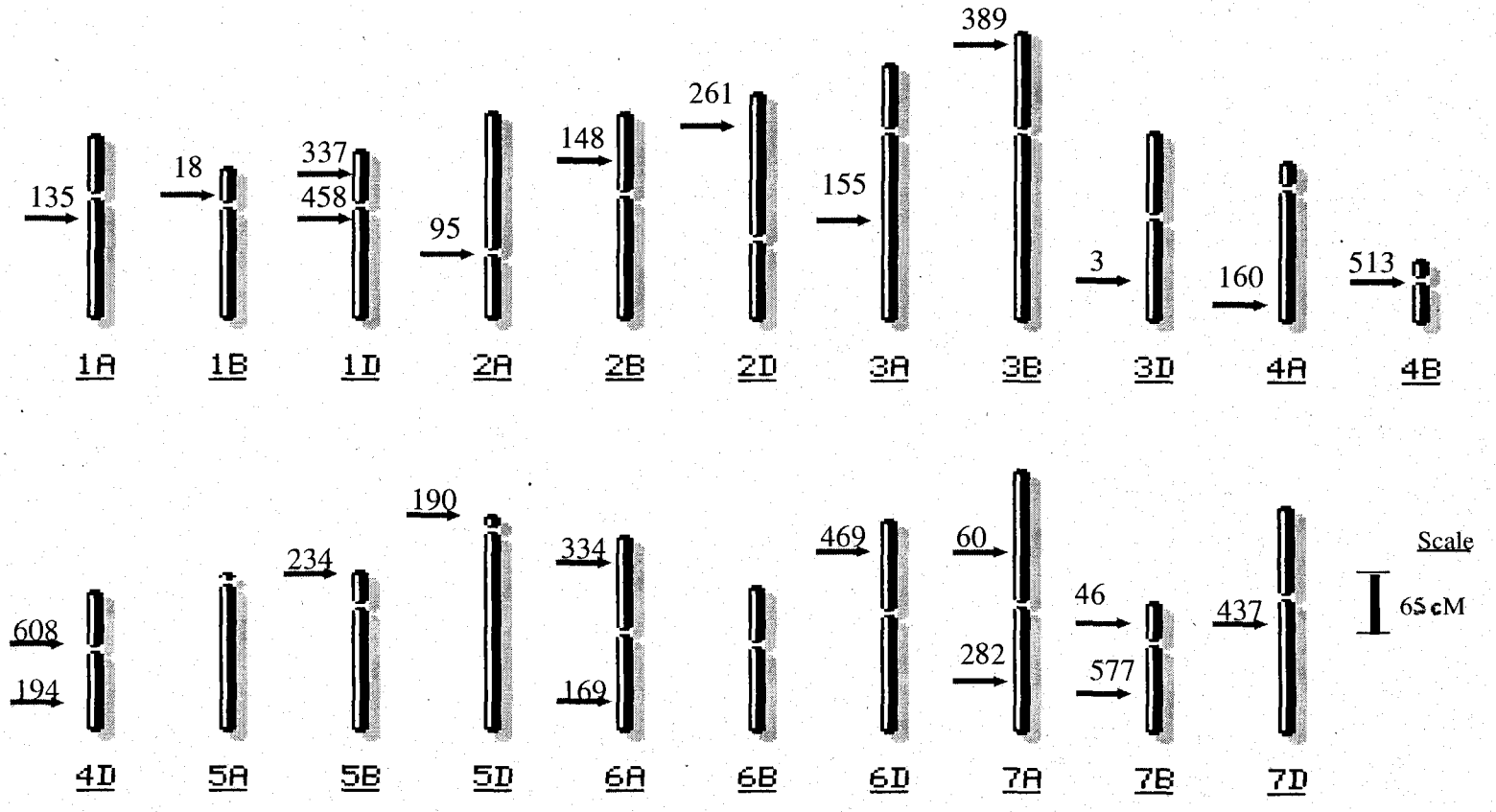

Figure 1. Map of 21 wheat linkage groups with approximate location of the 24 gwm markers used in this study.

(Greensboro, NC). The reverse primer for each of these markers was fluorescently labeled with TET (4,7,2',7'-tetrachloro-6-carboxyflourescein), FAM (6-carboxyflourescein), or Hex (4,7,2',4',5',7'-hexachloro-6-carboxyflourescein) for fluorescence-based detection.

PCR amplifications were carried out in an MWG Primus 96 thermocylcler in a 10 uL reaction mixture. Each reaction contained 10mM Tris-HCl (pH 9.0), 50mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.3 mM dNTPs, 0.03 U Taq polymerase (Promega), and 0.5 uM each of forward and reverse primers, with 100 ng of template DNA. The cycling parameters consisted of 3 minutes at 95 °C, followed by 45 cycles of 1 minute of 95 °C, 2 minutes at the pertinent annealing temperature and 1 minute at 74 °C. These cycles were then followed with a terminal extension of 10 minutes at 74 °C.

PCR products were diluted and sent to the Central Services Lab (CSL) at the Center for Gene Research and Biotechnology (Oregon State University) for fragment analysis using the ABI Prizm 3700® slab gel system and 3100® capillary system from Applied Biosystems (Foster City, CA). Allele sizes were determined using Genotyper® v 2.5.x Software (Applied Biosystems, Foster City, CA).

Data analysis

The nuclear markers are di-nucleotide repeats and therefore alleles were called based on two base pair differences in PCR product length. Chloroplast markers are single nucleotide repeats, and thus a single nucleotide difference in length should be considered a different allele. However, the CSL only guaranteed resolution to 0.5 base pairs. As this could result in some overlap in allele sizing, chloroplast alleles were called as with the nuclear markers on the basis of two base pair differences in PCR product length. While this reduces the precision of the diversity estimates, it increases the accuracy by reducing Type I error (calling alleles different that are actually the same). While some markers undoubtedly produce null alleles, all data points without allele sizes were treated as missing data, since null alleles and failed PCR cannot be distinguished. While this decreases the informativeness of markers that detect null alleles, it reduces the possibility of Type I error, increasing the accuracy of genetic diversity estimates using the entire marker set.

The number of alleles per locus, diversity estimates, number of taxon-specific alleles, and number of rare alleles (defined as occurring in less than 10% of the lines studied) were calculated with Microsat® v. 1.5d (Stanford, CA) and compared for the chloroplast SSR data alone, nuclear SSR data alone, and combined chloroplast and nuclear SSR data.

Three separate similarity matrices were produced for i) chloroplast SSR data, ii) nuclear SSR data, and iii) the entire data set using Microsat® v. 1.5d software, selecting the proportion of shared alleles statistic with 100 bootstraps. The matrix of the mean of bootstrap output was then analyzed by the SAHN cluster routine using the UPGMA (unweighted pair group method, arithmetic average) method of clustering using NTSYS software (Rohlfe 1992). The output was graphically represented as a dendrogram.

Results and Discussion

Chloroplast Diversity

Utility of Markers

Chloroplast markers for wheat have only recently been developed (Ishii et al. 2001), and have not yet been used to study wheat germplasm. The first objective of this study was to explore the effectiveness of these markers for diversity analysis of PNW wheat germplasm. Chloroplast SSR allele sizes for the genotypes studied here are presented in Appendix 2. Other chloroplast SSRs (cpSSRs) were tried (WCt16 and WCt24), but were found to be monomorphic at the two base pair level. These markers were eliminated from the overall analysis as they did not differentiate the germplasm and did not alter clustering patterns. Table 1 shows the number of rare alleles per locus and the number of taxon-specific alleles per locus. For the set of 15 chloroplast markers used, a total of 73 alleles were identified, with an average of 4.87 alleles per locus. This average was slightly higher than that of Ishii et al. (2000) who found an average of 4.13 alleles per locus for this set of cpSSRs. The exclusion of monomorphic markers from this study may account for the higher amount of polymorphism. For five of the markers, however, this study actually found slightly fewer alleles than previously reported. This may be related to scoring of alleles at a two base pair difference rather than a single nucleotide difference. Additionally, the plant material studied in Ishii et al. (2001) included more genetically divergent stocks,

Table 1. Allele frequencies for chloroplast simple sequence repeat markers.

Marker	*Total Alleles*	Rare Alleles [a]	Lines containing Rare Alleles (%)[b]	*Taxon-specific Alleles*	Lines with most common allele (%)
Wct1	3	1	1 (1)	1	91 (52)
Wct10	9	7	35 (20)	3	111 (64)
Wct11	7	5	20 (11)	2	94 (54)
Wct12	3	2	16 (09)	1	157 (90)
Wct13	4	2	27 (16)	0	132 (76)
Wct14	1	2	0 (1)	0	87 (50)
Wct15	14	11	72 (41)	3	33 (19)
Wct17	3	1	3 (2)	0	117 (67)
Wct18	2	0	0 (0)	0	151 (87)
Wct19	3	1	3 (2)	0	92 (53)
Wct22	3	2	11 (6)	1	164 (94)
Wct4	4	3	14 (8)	1	154 (89)
Wct6	6	4	15 (9)	0	93 (53)
Wct8	6	4	14 (8)	2	105 (60)
Wct9	3	0	0 (0)	0	113 (65)
Total	73	44	233 (9)	14	1694 (65)
Mean/ Locus	4.87	2.93	15.53	0.93	112.93

[a] Rare alleles are those present in less than 10% of the lines.

[b] Percentage is based on a total of 174 lines.

including several species, and thus a lower level of diversity is to be expected from this study. The set of markers yielded an average of 2.93 rare alleles per locus and 0.93 taxon-specific alleles per locus. Seven of these markers, however, revealed no taxon-specific alleles, and if these are eliminated, the average number of taxon specific alleles increases to 1.75 per locus. WCt10 and WCt15 yielded the highest number of both rare and taxon specific alleles, constituting over 40 percent of both the rare and taxon specific alleles of this pool.

The percentage of lines containing rare alleles and the percentage of lines containing the most common allele were identified for each marker (Table 1). Some markers, such as WCt22, WCt4, and WCt12 are essentially monomorphic, with 89 to 94% of the lines containing the most common allele. Other markers (WCt11, WCt6, WCt8, WCt1, WCt14, WCt19), however, were able to separate the majority of lines into two or more groups, since 60% or less of the lines contained the single most common allele. Thus, the markers that revealed a high percentage of lines represented by the single most common allele are less useful for the study of the major clustering events. The measurement of percentage of lines containing rare alleles indicates a useful marker because it reflects both the number of rare alleles yielded by that marker and their frequency in the population studied.

For the entire pool of 174 lines, 9% of the lines contained rare alleles and 65% of the lines contained the single most common allele (Table 1). The most useful markers

were those that revealed a high percentage of lines containing rare alleles with a low percentage represented by the single most common allele. These also revealed several alleles that were present in more than 10 % of the lines. The markers in this category were WCt1, WCt11, WCt14, WCt19, WCt6, and WCt15. While WCt10 revealed a high percentage of lines with rare alleles (20 %), 63% of the remaining lines are represented by a single allele. Thus, for the purposes of differentiating major groups, this marker was less useful than the others listed above.

Cluster Analysis

The second objective of this study was to analyze the cytoplasmic diversity available in PNW wheat breeding germplasm. These markers were able to distinguish the three plasmon types in this study (Figure 2a, b, c, and d). Analysis using UPGMA yielded three major groups, represented by the B type cytoplasm, D type cytoplasm, and barley (*Hordeum vulgare*) cytoplasm. This is in concordance with previous observations that these cpSSRs are able to distinguish between plasmon types of *Triticum* and *Aegilops* (Ishii et al. 2001). The barley group was most distantly related to the other two groups and contained five lines that had been included to test the efficacy of wheat cpSSR markers in this species. As no nomenclature has been established for this cytoplasm, it has been labeled here in concordance with its nuclear genome as H type cytoplasm.

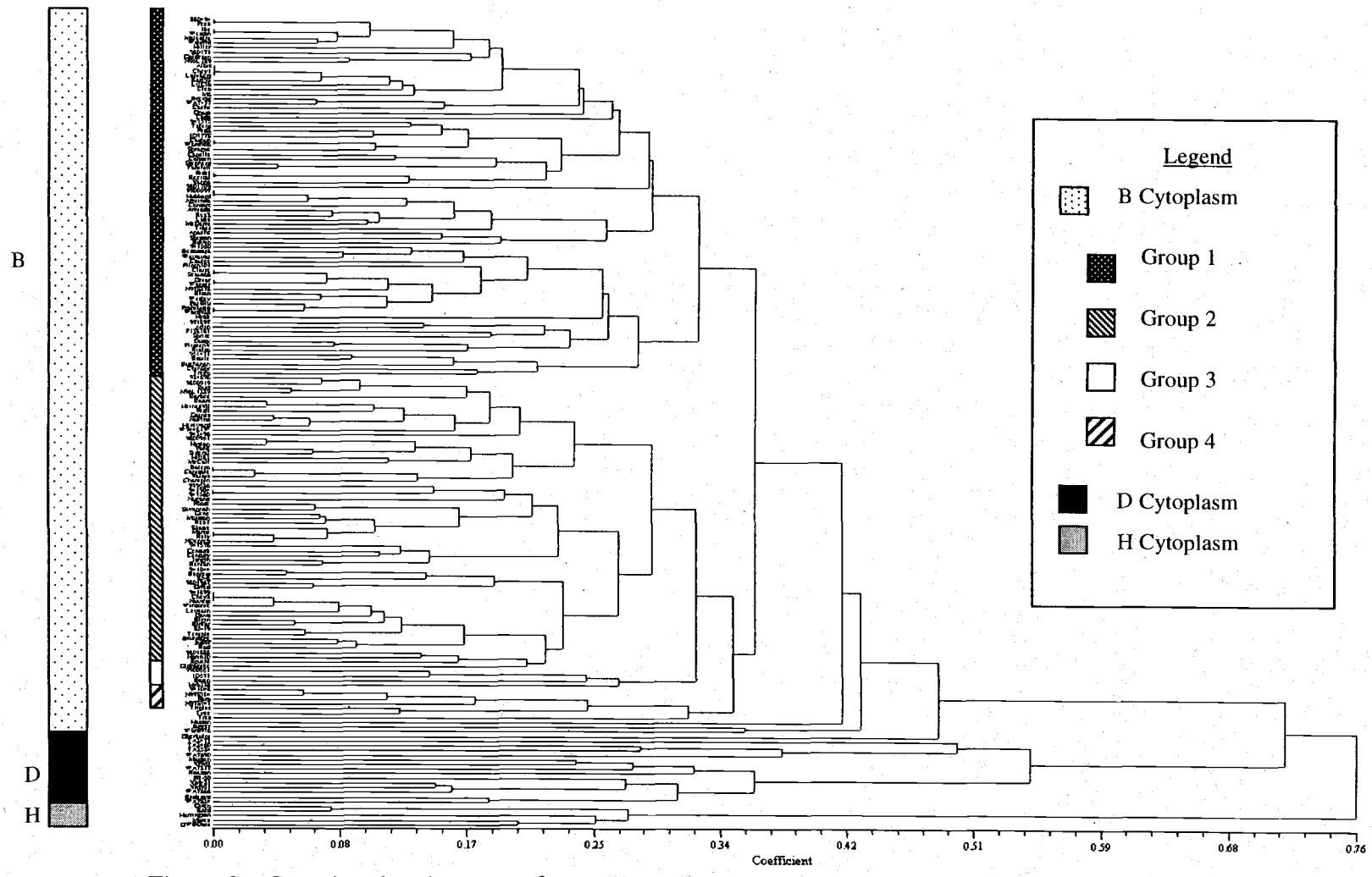

Figure 2a. Genetic relatedness tree from chloroplast SSR data, including B type cytoplasm, D type cytoplasm, and "H" type cytoplasm.

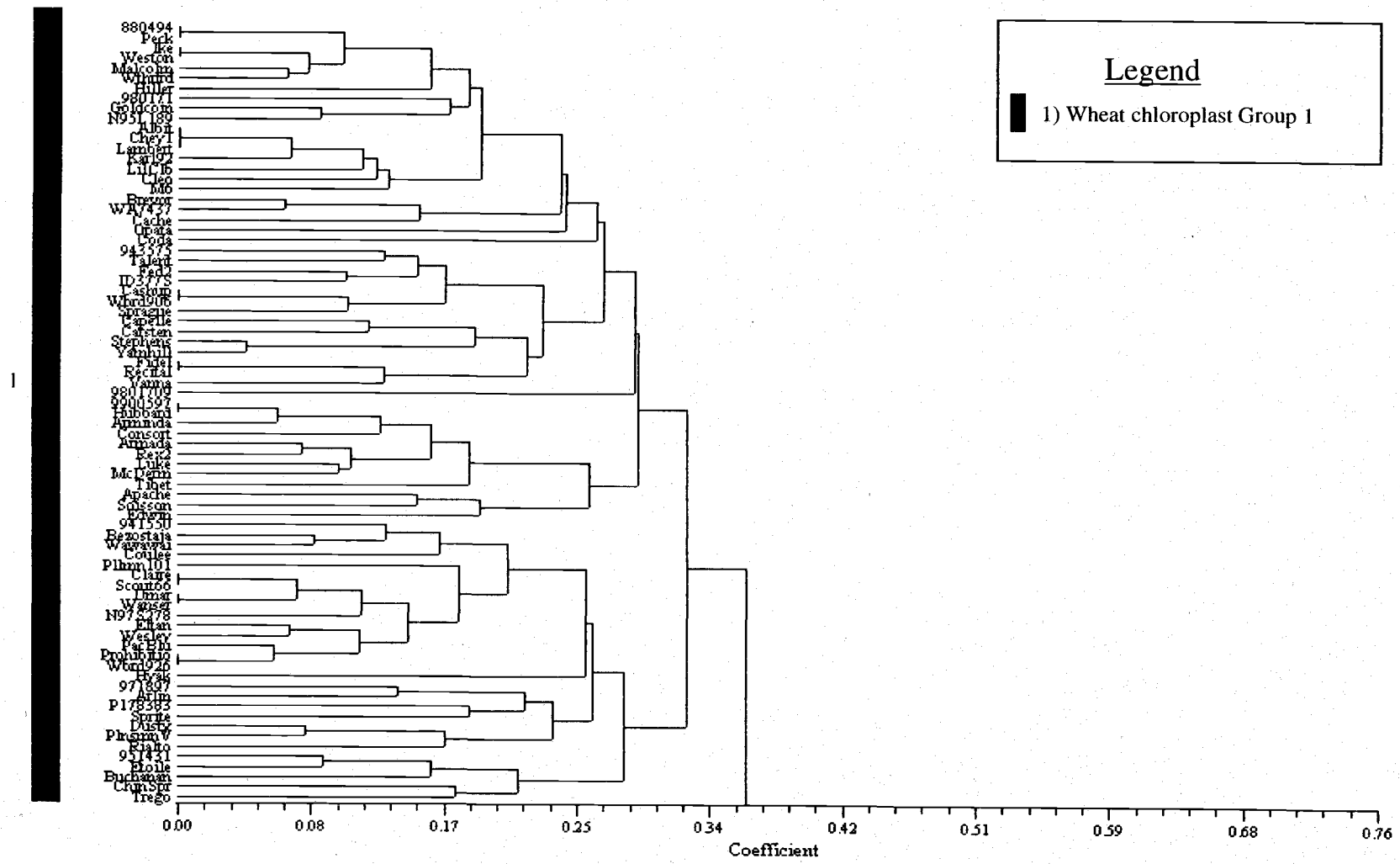

Figure 2b. Genetic relatedness tree from chloroplast SSR data, showing B type cytoplasm Group 1.

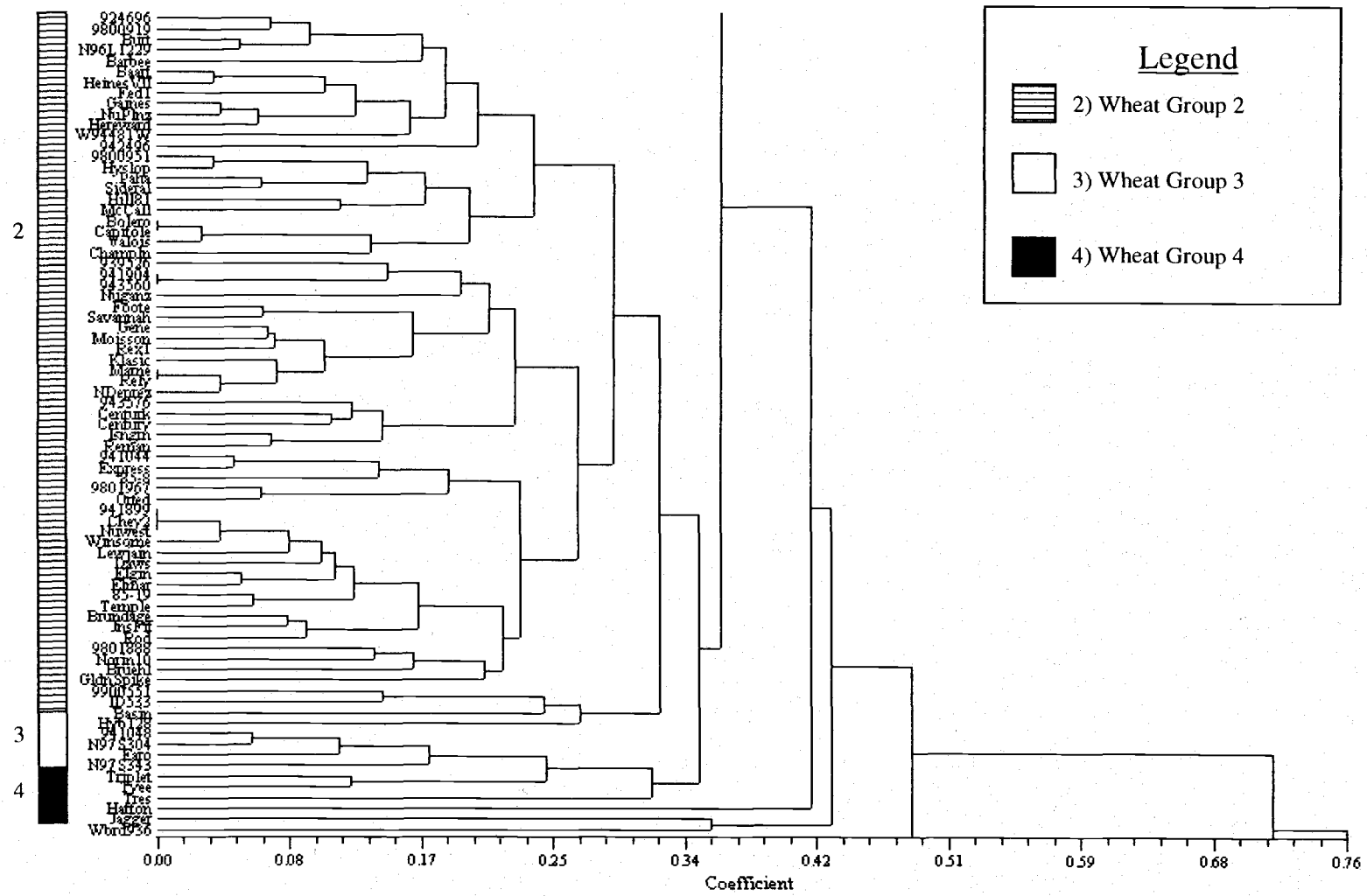

Figure 2c. Genetic relatedness tree from chloroplast SSR data, showing wheat B type cytoplasm Groups 2, 3, and 4.

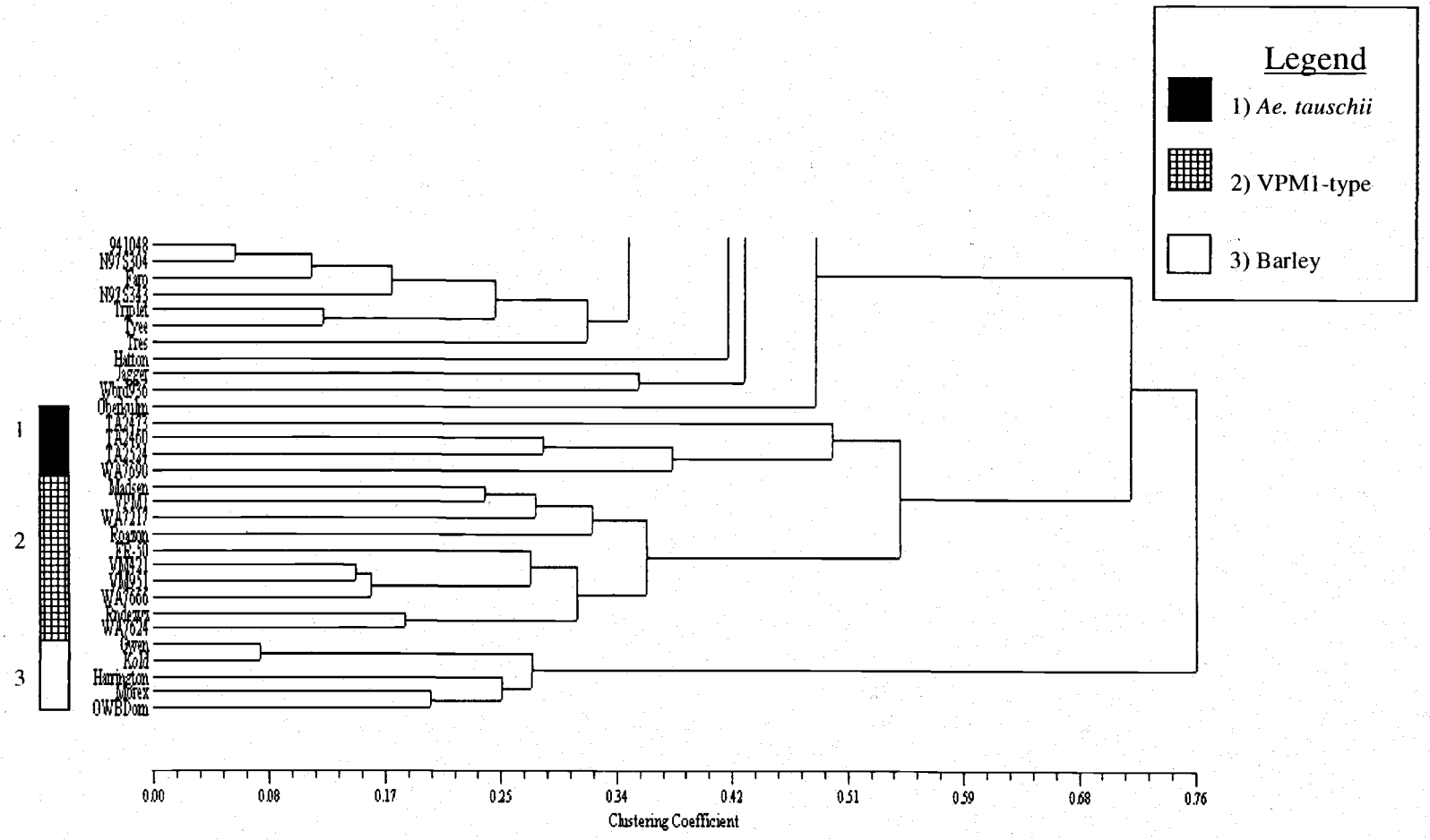

Figure 2d. Genetic relatedness tree from chloroplast SSR data showing non-wheat chloroplast types including barley and two types of D-type cytoplasm, *Ae. tauschii* and VPM1-type.

Within PNW wheat germplasm, there appear to be at least four major clusters of B type cytoplasm (Group 1 in Figure 2b, and Groups 2, 3, and 4 in Figure 2c). These clusters do not have any obvious biological or parental basis for their differentiation. The four groups cluster together at coefficient 0.44, but are distinct from genotypes with D type and barley cytoplasm by almost as much difference as is exhibited within the wheat group (0.36). Wheat chloroplast Groups 1 and 2 are two major groups that separate at coefficient 0.35. These connect to Group 3 at coefficient 0.42, which then connects to Group 4 at coefficient 0.44.

Synthetic hexaploid wheats, produced by crossing durum wheat (*T. turgidum*, 2n = 4x = 28, AB genomes) with *Ae. tauschii* (2n = 2x = 14, D genome), have been shown to have potentially useful genetic variation for wheat improvement (del Blanco et al. 2001). M6 is a synthetic hexaploid wheat included in this study. It clustered with genotypes in Group 1 of wheat (Figure 2b). This was expected as durum wheat, which has B type cytoplasm, was used as the maternal parent in its development.

In addition to four major wheat cytoplasmic groups, three minor distinct groups were found. One group was represented by the cultivars Hatton, Jagger and Westbred 936, and Oberkulmer. Based on pedigree information, Hatton possesses the cytoplasm of an Iranian landrace (PI 142522). The nature of the cytoplasm of Jagger and Westbred 936 is unknown based on the available information. This study suggests that these cultivars share a common B type cytoplasm lineage that is quite distinct from the

majority of the cultivars studied. At coefficient 0.48, the line Oberkulmer connects with the other groups of the wheat B cytoplasm group (Figure 2d). This line is a Western European landrace of spelt wheat. Spelt wheat is considered a subspecies of *T. aestivum* (ssp. *spelta),* and thus was expected to have a distinct plastome from other wheat types.

The D type cytoplasm also constituted a group that was separate from either wheat or barley (Figure 2d). It is related to B type cytoplasm at the coefficient level of 0.79, intermediate between the relatedness level of wheat B plasmon type and barley H plasmon type, as would reflect genetic relatedness between the *Aegilops* and *Triticum* genera, which are more closely related to one another than either is to barley. This cluster is then subdivided into two groups at the coefficient level of 0.64, and contains the highest level of within-group variation. In fact, the two clusters may represent two distinct plastome types of *Aegilops*, one containing four lines of classic D type plastome from *Ae. tauschii* and one containing 10 lines that putatively have cytoplasm from *Ae. ventricosa.* Ishii et al. (2001) found that these cpSSR markers were sufficient to differentiate between various other species of *Triticum* and *Aegilops* (such as *Ae. speltoides, Ae. squarrosa, T. araraticum, T. dicoccoides, T. dicoccum, T. aestivum, T. spelta,* and *T. macha*). Thus it is not surprising that these markers differentiated the various cytoplasmic types in this study.

A cluster representing VPM1-related cytoplasm (Group 1 in Figure 2d) was identified which consists of ten wheat lines from both Europe and the PNW with resistance to the fungal disease strawbreaker foot rot (eyespot), caused by *Pseudocercosperella herpotrichoides*. These lines have this resistance from the breeding line VPM1, which was developed from a cross of *Ae. ventricosa*/*T. aestivum*. Pedigree information for some of these lines can be found in Appendix 4. The maternal parent of VPM1, based on this analysis was *Ae. ventricosa*, thus VPM1 and any offspring for which it served as the maternal parent contain *Ae. ventricosa* type cytoplasm. This group is distinct from, but related to, the chloroplast type of *Ae. tauschii* (Group 2 in Figure 2d). Included in the group of VPM1 descendants is Madsen, which is a widely grown variety in the PNW. This finding demonstrates the successful alloplasmic lineage of wheat varieties.

Rendezvous is a Western European line that also has resistance to eyespot that was derived from VPM1 (Angus 2001). It appears to have *Ae. ventricosa* cytoplasm, which indicates that VPM1 was a maternal parent in its development. Other European lines that appear to have *Ae. ventricosa* cytoplasm are Roazon and FR-50.

Numerous lines derived from VPM1 do not cluster with the D type cytoplasm. Coda, Temple, and Weatherford all had VPM1 as a paternal parent, and thus have retained B cytoplasm. The pedigree of Hyak indicates VPM1 as the maternal parent, yet it appears in Group 1 (Figure 2b) of the B cytoplasm group. This suggests that the

actual pedigree for Hyak should indicate a different direction of crossing. Tyee should be the maternal parent, and VPM1/Moisson 421 the paternal parent, either in the original cross or the backcross. This would explain why Hyak clusters with the B cytoplasm rather than with all of the other lines descended from VPM1. WA 7690, which is listed as being a VPM1 descendent, and which contains eyespot resistance, however, clusters with the *Ae. tauschii* rather than with Group 2 of the D cytoplasmic lines. The reasons for this are unclear.

Barley lines were included in this study to test the efficacy of wheat chloroplast markers in this species. All markers in this group were able to amplify barley chloroplast DNA. These lines clustered separately from both D cytoplasm and B cytoplasm types and served to root the dendrogram` at a clustering coefficient of 0.92 (Figure 2d). They were more closely related to one another than are the wheat lines in Groups 1 through 4, since the highest clustering coefficient within the barley group is 0.31.

Conclusions

The first objective of this study was to examine the usefulness of these markers in this germplasm. The 15 wheat chloroplast markers used in this study were able to differentiate three cytoplasmic types- B type, D type, and barley. They revealed at least five plastomic types within the wheat plasmon group studied. These markers

were also able to distinguish spelt wheat from common wheat, but were unable to differentiate durum wheat from hexaploid wheat.

Two markers (WCt10 and WCt15) accounted for over 40% of the rare and taxon-specific alleles. Several other markers were found to be particularly useful for studying the chloroplast diversity of PNW wheat breeding germplasm because they revealed several rare alleles and were also able to segregate these lines into two or more distinct groups (WCt11, WCt6, WCt8, WCt1, WCt14, and WCt19).

The second objective of this study was to analyze the chloroplast diversity available. Five plastomic types were found within the B cytoplasm group. In addition, several of the lines in PNW wheat breeding germplasm appear to contain the cytoplasm of *Ae. ventricosa* rather than that of wheat, including one of the most commonly grown cultivars in the region, Madsen. This is an example of inadvertent widening of the cytoplasmic diversity of breeding germplasm by introduction of eyespot resistance from VPM1 material. Additionally, nuclear/cytoplasmic interactions may have effects on desired traits and thus the presence of this alien cytoplasm in wheat lines is significant. Thus, alloplasmic effects need to be further studied.

Nuclear Diversity

Utility of Markers

Complete allele sizing data from nuclear SSRs are shown in Appendix 3. The first objective of this study was to determine the usefulness of these markers in PNW wheat breeding germplasm. For this set of 24 nuclear markers, a total of 268 alleles were identified. The number of alleles per locus ranged from 4 for gwm608 to 30 for gwm282, with an average of 11.2 per locus (Table 2). Previous studies have shown a range of average alleles per locus for SSR markers from 4.2 (Stachel et al. 2000) to 18.1 (Huang et al. 2002). Genetic diversity of 500 European lines was assessed using 19 SSRs (Roeder et al. 2002), including 12 of those used in this study, and the average alleles per locus was 10.3. This is in contrast to the 18.1 alleles per locus reported by Huang et al. (2002), from a survey of accessions from the germplasm bank at the Institute for Plant Genetics and Crop Plant Research (IPK), Gatersleben (Germany). The average number of alleles per locus from this study was more similar to that of European germplasm than that of the germplasm bank. As both the current study and that of Roeder et al. (2002) involved a particular germplasm group consisting of predominantly winter wheat, this result was expected. The slightly higher measurement obtained from the current study is most likely due to the number of markers, the particular markers selected, and the addition of *Ae. tauschii*, M6, Oberkulmer and other unrelated wheats.

Table 2. Allele frequencies for nuclear simple sequence repeat markers.

Marker	*Total Alleles*	Rare Alleles [a]	Lines containing Rare Alleles (%)[b]	*Taxon-specific Alleles*	Lines with most common allele (%)
Xgwm3	7	4	15 (9)	2	63 (37)
Xgwm18	9	7	26 (15)	2	73 (43)
Xgwm46	18	13	32 (18)	6	49 (29)
Xgwm60	11	9	43 (25)	3	72 (42)
Xgwm95	7	4	13 (8)	0	62 (36)
Xgwm135	8	6	13 (8)	1	58 (34)
Xgwm148	7	4	14 (8)	1	41 (24)
Xgwm155	8	6	34 (20)	2	73 (43)
Xgwm160	7	6	52 (30)	1	79 (46)
Xgwm169	9	7	63 (37)	0	23 (14)
Xgwm190	14	11	63 (37)	4	46 (27)
Xgwm194	10	6	17 (10)	2	43 (25)
Xgwm234	12	7	28 (16)	3	30 (18)
Xgwm261	11	9	36 (21)	4	93 (54)
Xgwm282	30	27	88 (52)	10	23 (14)
Xgwm334	9	5	32 (19)	2	39 (23)
Xgwm337	15	13	75 (44)	3	64 (37)
Xgwm389	14	11	57 (33)	3	41 (24)
Xgwm437	13	9	47 (28)	1	43 (25)
Xgwm458	9	5	13 (8)	2	70 (41)
Xgwm469	13	9	36 (21)	3	38 (22)
Xgwm513	6	1	8 (5)	0	44 (26)
Xgwm577	17	14	59 (35)	6	30 (18)
Xgwm608	4	1	4 (2)	0	66 (39)
Total	268	194	868	61	1263
Mean	11.2	8.1	36.2 (20)	2.5	52.6 (30)

[a] Rare alleles are those present in less than 10% of the lines. [b] Percentage is based on 171 lines.

Fourteen of the markers in this study were also in a recent study by Huang et al. (2002). Each of these markers, except gwm458, yielded a lower number of alleles per locus in this study than previously reported. The study by Huang et al. (2002) involved four times as many genotypes, including lines from 68 countries of five different continents. It is not unexpected that a study of a set of germplasm adapted to one particular region would exhibit less allelic variation than the broader international germplasm study.

The markers in this study showed an average of 8.08 rare alleles per locus and 2.54 taxon-specific alleles per locus. Five of the markers yielded no taxon-specific alleles. Gwm282 yielded both the highest number of taxon specific alleles and the highest number of alleles for a single marker (30). The distribution of alleles was compared with that reported by Huang et al. (2002). The distribution of alleles was not similar to those reported for the majority of markers. This study revealed fewer normally distributed markers and more randomly distributed markers than observed by Huang et al (2002). These results suggest that allelic distribution, and perhaps informativeness in general, is specific to the set of markers *and* populations studied rather than a function of the individual markers themselves.

Cluster Analysis

Major Clusters

The third objective of this study was to explore the nuclear diversity of this germplasm and to determine the biological significance of nuclear clustering patterns. Pedigree information was used to explain and characterize major clusters as based on nuclear SSR data (Appendix 4). The dendrogram from nuclear SSR data was inspected to locate clustering branches with several lines that had coefficients higher than 0.74. Five major clusters (Figure 3) were identified: Group 1 was represented by PNW soft white wheat clusters; Group 2 includes European-derived and related red wheat cultivars; cultivars in Group 3 a less distinct cluster or more distantly-related lines; Group 4 contains hard wheat cultivars from the Great Plains; and Group 5 includes lines used as genetic stocks, synthetics, and other distantly related cultivars. Group 1 and Group 2 cluster at a coefficient of 0.74. Group 4 then joins Groups 1 and 2 at coefficient 0.81. There were several subclusters within each of these major clusters. Lines in Group 3 clustered very loosely with the major groups (clustering coefficients higher than 0.84). The primary biological division for the major groups appears to be regional adaptation. This is consistent with a previous report of a wheat diversity study using SSR data (Roeder et al. 2002). As particular market classes are grown in different regions in the U.S., the clusters also appear to differentiate, although less distinctly, on market class.

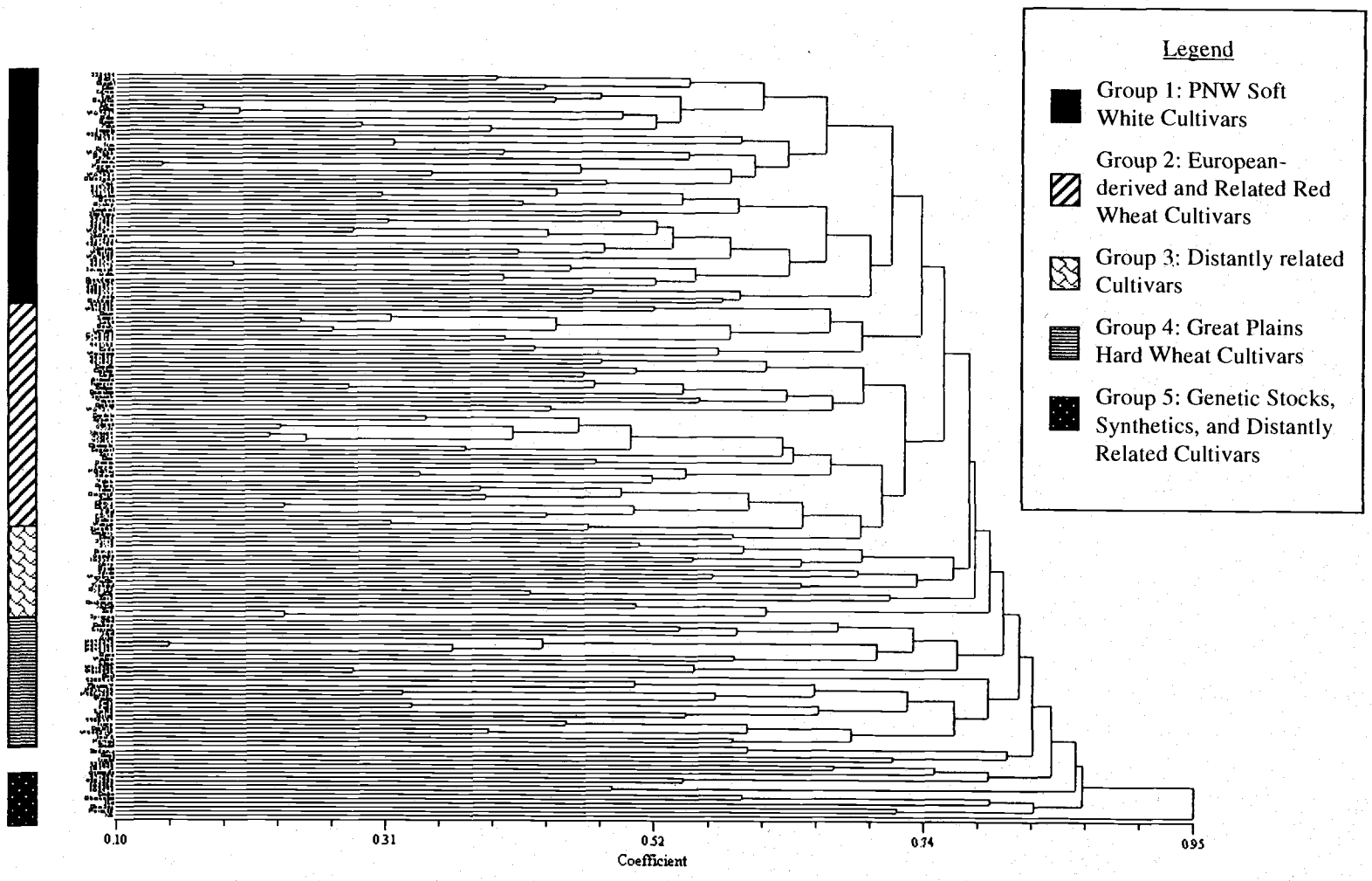

Figure 3a. Genetic relatedness tree from nuclear SSR data with major clusters.

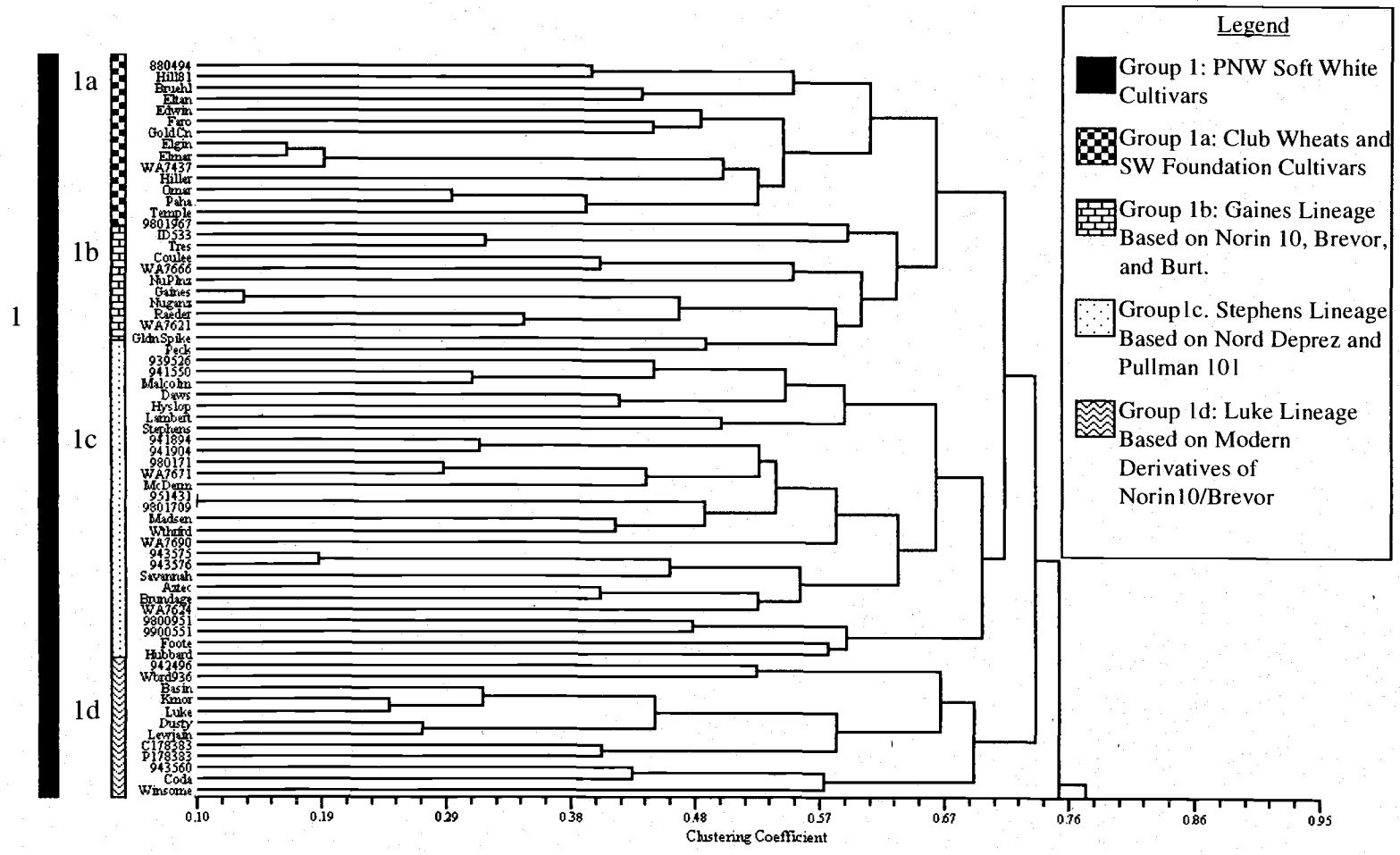

Figure 3b. Genetic relatedness tree from nuclear SSR data. Group 1: PNW Soft White Cultivars

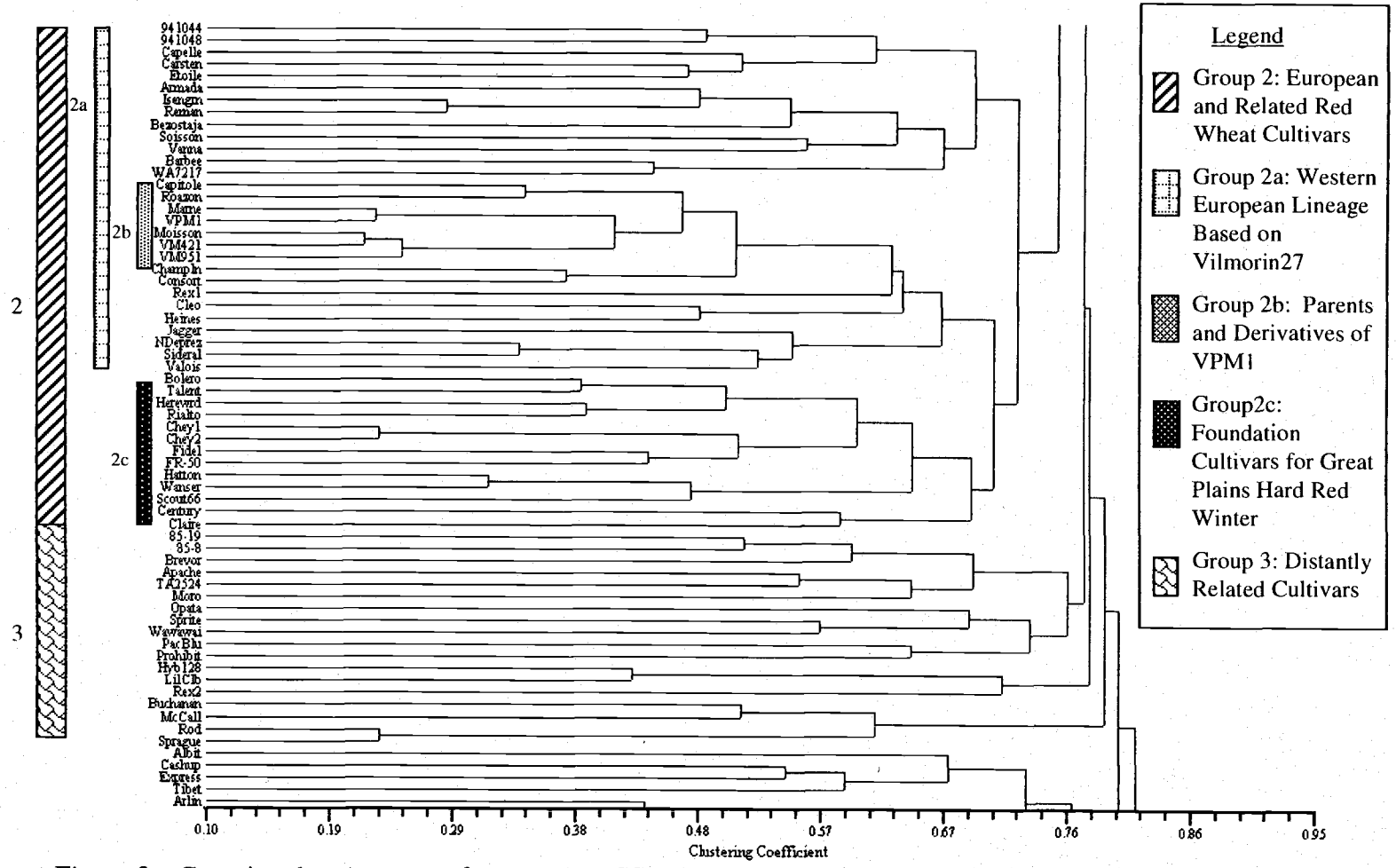

Figure 3c. Genetic relatedness tree from nuclear SSR data. Group 2: European and Related Red Wheat Cultivars and Group 3: Distantly Related Cultivars.

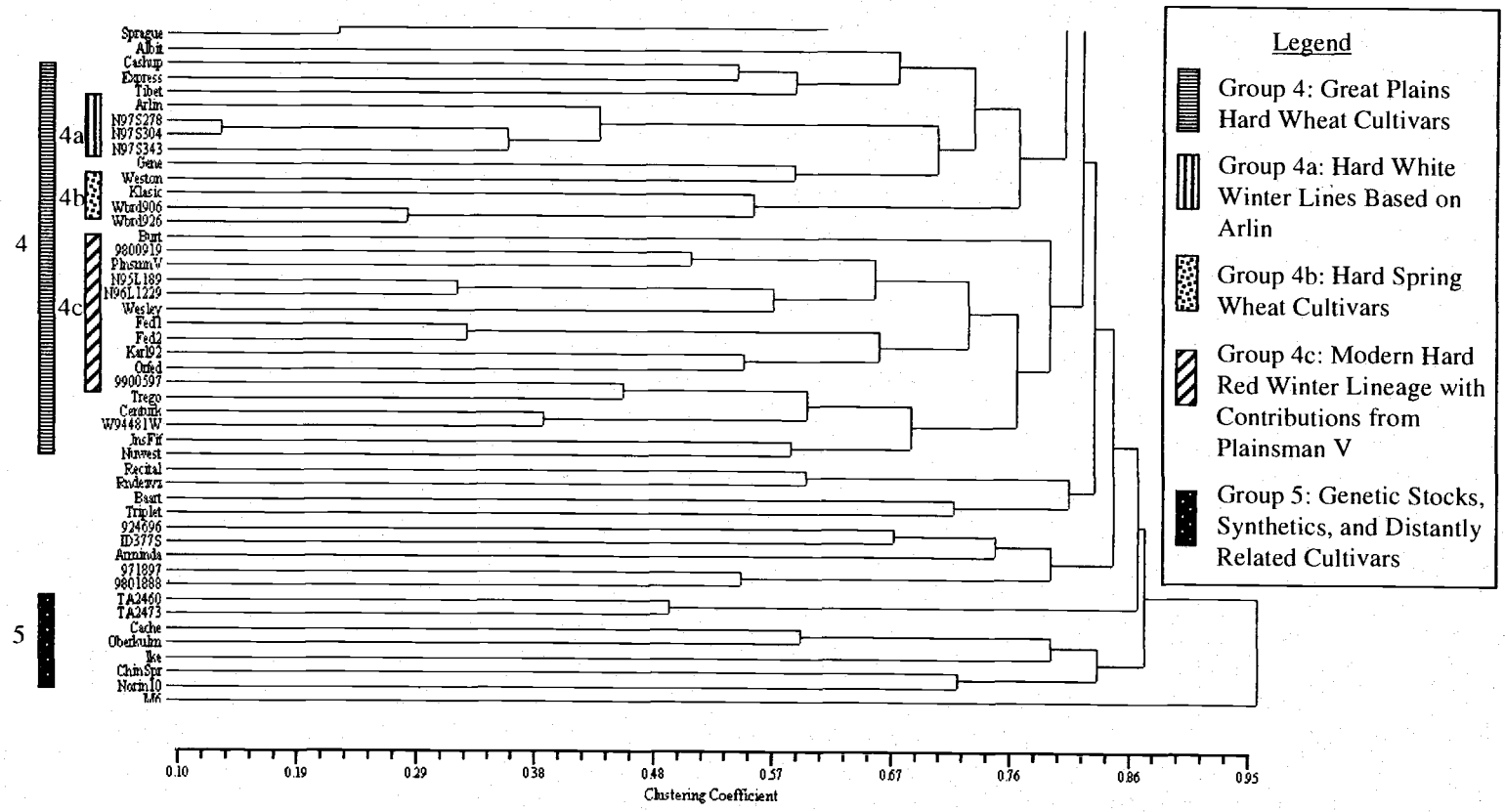

Figure 3d. Genetic relatedness tree from nuclear SSR data. Group 4: Great Plains Hard Wheat Cultivars and Group 5: Genetic Stocks, Synthetics, and Distantly Related Cultivars.

AFLP analysis by Barrett and Kidwell (1998) suggested that divisions between market classes (hard vs. soft or red vs. white) within growth habit (winter, spring, or facultative) were not statistically supportable by clustering associations. In this study, while the *primary* factor in clustering appears to be regional, market class contributes to clustering within regional groups.

Group 1: Pacific Northwest soft white wheat cultivars

The first major group (converging at a clustering coefficient of 0.72) consists of soft white winter wheat cultivars from the PNW (Cluster 1 in Figure 3b). This group is further divided into four subsets on the basis of shared parental lines. Group 1a (coefficient 0.61) is composed of lines of club wheat and parental lines that provide much of the genetic foundation of the soft white class. Group 1b consists of lines closely related to Gaines with lineage based on parental lines Norin 10, Brevor, and Burt. This group includes the first major semi-dwarf varieties of wheat in the PNW released in the late 1960's. Group 1c (coefficient 0.71) cultivars were defined by commonalities with Stephens, and based on parental lines Nord Deprez and Pullman 101. Group 1d, which joins the clusters at coefficient 0.72, is linked through association with Luke, and represents the modern derivatives of Norin 10/ Brevor Group. Groups 1a and 1b cluster the most closely together (coefficient 0.67). Lines in Group 1a were used as parents in development of many lines in Group 1b.

Group 1a: Club wheat and soft white foundation cultivars

The first group (Group 1a in Figure 3b) is represented predominantly by club wheats, and includes such major cultivars as Elgin, Omar, Edwin, Hiller, and Tres. This Group also includes lines that are important progenitor stocks, such as Goldcoin, and modern variety clubs like Tres and Elgin, which have been used in the development of other cultivars. Additionally, several of them are parent/offspring duos (see pedigree information in Appendix 4). White club wheat was previously reported as the only category of winter wheat that consistently clustered together (69% of bootstrap trees) using AFLP data (Barret and Kidwell 1998).

Six club lines (Barbee, Coda, Albit, Hybrid 128, Little Club, and WA 7621) failed to be associated with Group 1a, and were dispersed throughout the other clusters. Little Club is a spring wheat, thus it is not unexpected that it segregated separately from the winter club group (in Group 3 in Figure 3c). It is also a major parent of Hybrid 128, which is in turn a major parental contributor of Albit (Group 4 in Figure 3d). WA 7621, Coda, and WA 7217 are all club wheats that have been bred for resistance to strawbreaker foot rot (eyespot) with parental contributions of common wheat and VPM1. They cluster with other of VPM1-related lines (Group 2b in Figure 3c). Barbee, also derived from a club by common wheat cross (but not VPM1 related), and its progeny WA 7217 were distinct and clustered more closely with common wheats than with the other club lines.

Group 1b: Gaines lineage based on Norin 10, Brevor, and Burt

The cluster most closely related to club wheats (Group 1b in Figure 3b) includes lines related to Gaines, or its parents Norin 10, Brevor, and Burt. This group includes the first PNW semi-dwarf varieties and the genetic background into which the semi-dwarf trait was introduced. These cluster closely with the club wheats because soft white common cultivars such as Gaines, Nugaines, Coulee, and Raeder have several common parental lines, including Goldcoin, which is an important ancestor of modern club wheats.

Group 1c: Stephens lineage based on Nord Deprez and Pullman 101

Groups 1a and 1b join a large cluster of soft white winter (SWW) wheat varieties at coefficient 0.70. This cluster (Group 1c in Figure 3b) is characterized by relationship to Stephens, a major SWW cultivar, and its progenitors Nord Deprez and Pullman 101. Stephens was released in 1978 by Dr. Warren Kronstad at Oregon State University. It has been a major cultivar in PNW production for over two decades. It clusters with its progeny lines such as ID 533, Lambert, Malcolm, Edwin, Brundage, and WA 7624. This cluster includes other lines based on Nord Deprez and Pullman 101, Hyslop, McDermid, and Hill 81 and their more recent offspring WA 7671, WA 7690, Madsen, and Weatherford. Also clustering in this group are 12 Oregon experimental lines that are largely based on parents from this cluster.

Group 1d: Luke lineage based on modern derivatives of Norin 10/Brevor

The final subset of the PNW cluster includes Luke and modern PNW cultivars related to Luke. These lines coalesce at coefficient 0.69. The majority of these cultivars (Luke, Daws, Lewjain, and Dusty) were released in the late 1970's and early 1980's from Washington. They represent the second generation of semi-dwarf improvement in Washington and are related through the progenitor lines Norin 10, which is the semi-dwarf gene donor, and Brevor. These lines, developed in Washington, are related to the Group 2b and share many adaptive characteristics.

Group 2: European and related red wheat cultivars

Group 2 was characterized by a grouping of developed European lines (Figure 3c). Cultivars from Western Europe have many similar adaptive characteristics to PNW soft whites and have been important parents in PNW varietal development efforts. Oregon State University has initiated new germplasm exchange and introgression efforts based on Western European material. The European lines included in this study coalesce at coefficient 0.74. This cluster was further divided into subgroups related to parental contribution; Group 2a includes all of the lines except those in Group 2d, and is defined by Western European cultivars with lineages based on Vilmorin 27, Group 2b (coefficient 0.47) consisting of a subset of lines related to VPM1, and Group 2c (coefficient 0.72) that contains a set of foundation cultivars used in development of the Great Plains hard red winter wheat.

Group 2a: Western European lineage based on Vilmorin 27.

Group 2a (Figure 3c) includes many Western European cultivars and two OSU experimental lines. These OSU lines have parental contribution from germplasm from the Netherlands. Within this cluster are several French cultivars derived from Vilmorin 27, including major cultivars Capelle Deprez, Nord Deprez, Isengrain, Talent, and Champlein. Jagger, a Great Plains hard red winter wheat is included in this cluster. It is derived from Stephens, which has parental contributions from Nord Deprez. Capitole and Moisson are both derived from Vilmorin 27 and another major French parental line, Etoile de Choisy, which is also included in this cluster. Renan and WA 7217 both have Norin 10 and Brevor in their backgrounds, as well as contributions from Vilmorin 27. This commonality, along with the Nord Deprez link, may explain why this group clusters more closely with PNW soft whites than does the Group from the U.S. Great Plains.

Group 2b: Parents and derivatives of VPM1

Within Group 2a, there is a tight cluster of lines that are closely related to VPM1 and its progeny, which is identified as Group 2b (Figure 3c). Marne was the common wheat parental line of VPM1 and clusters closely with it, at a coefficient of 0.24. Moisson is SWW wheat that is susceptible to strawbreaker foot rot (eyespot), an important fungal disease caused by *Pseudocercosporella herpotrichoides*, and was used in crosses with VPM1 to improve the yield and adaptive characteristics. VPM1 has been an important parent in PNW variety development, contributing eyespot

resistance into PNW wheat breeding germplasm. The two major lines derived from these crosses (VPM1/Moisson 421 and 951) both cluster more closely to Moisson (coefficient 0.25) than to VPM1 (coefficient 0.41) likely due to selection for the adaptive characteristics of Moisson.

Group 2c: Foundation cultivars for Great Plains hard red winter

Group 2c includes major cultivars and parents of Great Plains hard red winter wheat. Although the relationship with European materials is less obvious, these cultivars have origins in European landrace material, particularly from Eastern Europe, through Cheyenne, which is a selection from the landrace Crimean. Scout 66, Wanser, Hatton, and Century are all Cheyenne-derived.

Group 3: Distantly related cultivars

Lines in Group 3 (Figure 3c) appear to have no obvious basis, whether through pedigree or regional adaptation, for clustering together. They coalesce at a coefficient of 0.80. This high coefficient value within the cluster suggests that they are generally not closely related.

Group 4: Great Plains hard wheat cultivars

Group 4 (Figure 3d) is characterized by Great Plains hard wheat cultivars. Group 4 joins the previous sub-groups at coefficient 0.82. Within this diverse group, three closely related sub-groups stand out. Group 4a (coefficient 0.44) is a set of four lines

of hard white winter wheat that includes Arlin and progeny derived from single crosses with Arlin. Group 4b (coefficient 0.55) is a small cluster of three hard spring cultivars. This association is notable because of the few spring cultivars included in this study. Group 4c contains Plainsman V and hard red wheats that have Plainsman V or Kansas or Nebraska germplasm as common parents (N95L189, N96L1229, Wesley, Karl 92).

Group 5: Genetic stocks, synthetics, and distantly related cultivars

A number of genetic stocks (M6, Norin 10, Chinese Spring, Ike, Oberkulmer, Cache, and two *Ae. tauschii* accessions) showed little relationship (greater than coefficient 0.85) to the other groups (Group 5 in Figure 3d). M6 (the most different wheat in this study with a coefficient of 0.95) is a synthetic wheat derived from a *T. turgidum/ Ae. Tauschii* cross. Norin 10 is the original donor of semi-dwarf genes into PNW cultivars. Chinese Spring is an important stock for cytogenetic research. Ike is a HRW line developed in Kansas with a different genetic base than other Great Plains wheats included in this study. Oberkulmer is a spelt wheat, which is a different subspecies of wheat than common hexaploid bread wheat. Finally, Cache is an older (1944) hard red winter wheat developed in Utah, that was based on Kansas hard red parentage.

Conclusions

The markers in this study effectively clustered PNW wheat germplasm into groups with commonalities based on parentage and origin. Approximately 20% of the lines

were grouped in ways not obvious by parental relationships or shared adaptation. Lines generally clustered with close relatives, and the predominant divisions appear to be based on regional adaptation (PNW vs. Europe vs. Great Plains). Additionally, although lines do not segregate conclusively on the sole basis of market class, the clustering pattern suggested that a commonality was market class. For instance, the European-derived group actually contains several U.S. hard red winter wheats in addition to the European hard red cultivars. Market class appears to be a shared characteristic within this group. This cluster of predominantly hard red wheats, however, is more closely related to PNW soft whites than to the other hard red wheats from the Great Plains, indicating that market class alone is not the primary segregating factor.

Overall, the amount of polymorphism, alleles per locus, and allelic distribution indicated by these markers indicate that the PNW wheat breeding germplasm contains a level of diversity on par with that of the European cultivars analyzed at the IPK (Roeder et al. 2002). The divergence of PNW soft white wheat from European-derived lines suggests opportunity to exploit European germplasm for further improvement of PNW cultivars.

Combined Nuclear/ Chloroplast Analysis

Combined Clustering Patterns

The final objective of this study was to answer specific questions regarding the diversity of the PNW wheat breeding germplasm using genetic diversity estimates from the combined data sets. Clustering patterns for the entire set of markers, nuclear and chloroplast, were investigated for 165 lines included in both studies (Figure 4a). The structure of this combined dendrogram was compared to the individual dendrogram relationships yielded by chloroplast versus nuclear SSRs. The coefficients were intermediate (major branch points starting around 0.58) between those indicated by chloroplast (major branch points beginning at 0.32) and nuclear (major branch points starting around 0.74) data. The primary distinction in the combined tree is the separation of cultivars based on B versus D cytoplasmic types, which are labeled on Figure 4a. Within the B cytoplasmic type, the club wheats form a cluster, as well as the Stephens-related wheats (Group 1 and 2 respectively on Figure 4b). At coefficient 0.58, there is a large cluster that resembles the nuclear cluster formed by the European and PNW wheat together (Group 1 on Figure 4a), but the subclusters within that cluster do not separate into concise, pedigree based clusters such as those formed when nuclear data was analyzed alone. However, lines that are very closely related via pedigree (Appendix 4), such as sister lines or parent/offspring duos continue to cluster together.

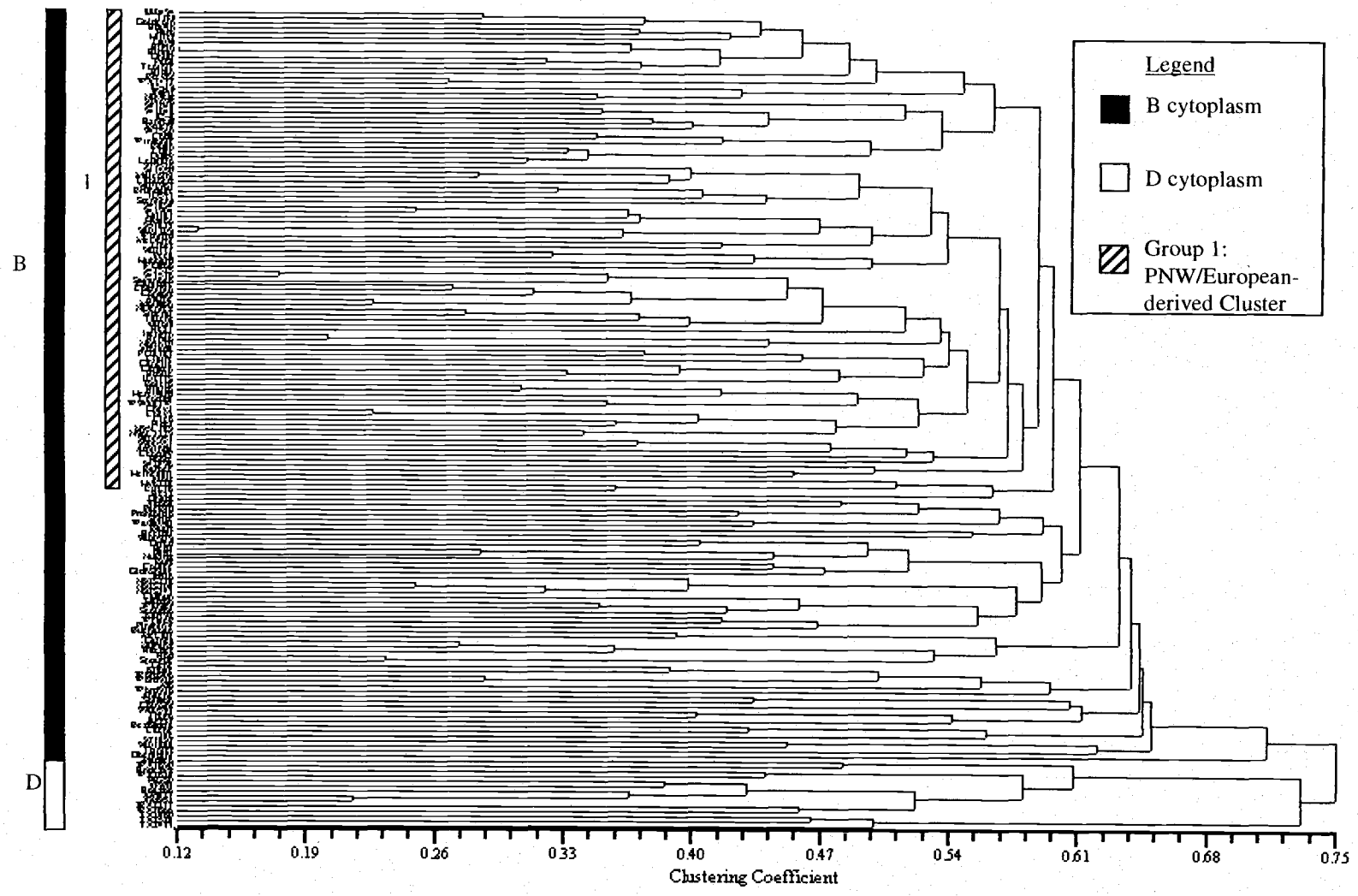

Figure 4a. Genetic relatedness tree of PNW wheat breeding germplasm based on nuclear and chloroplast SSR data combined.

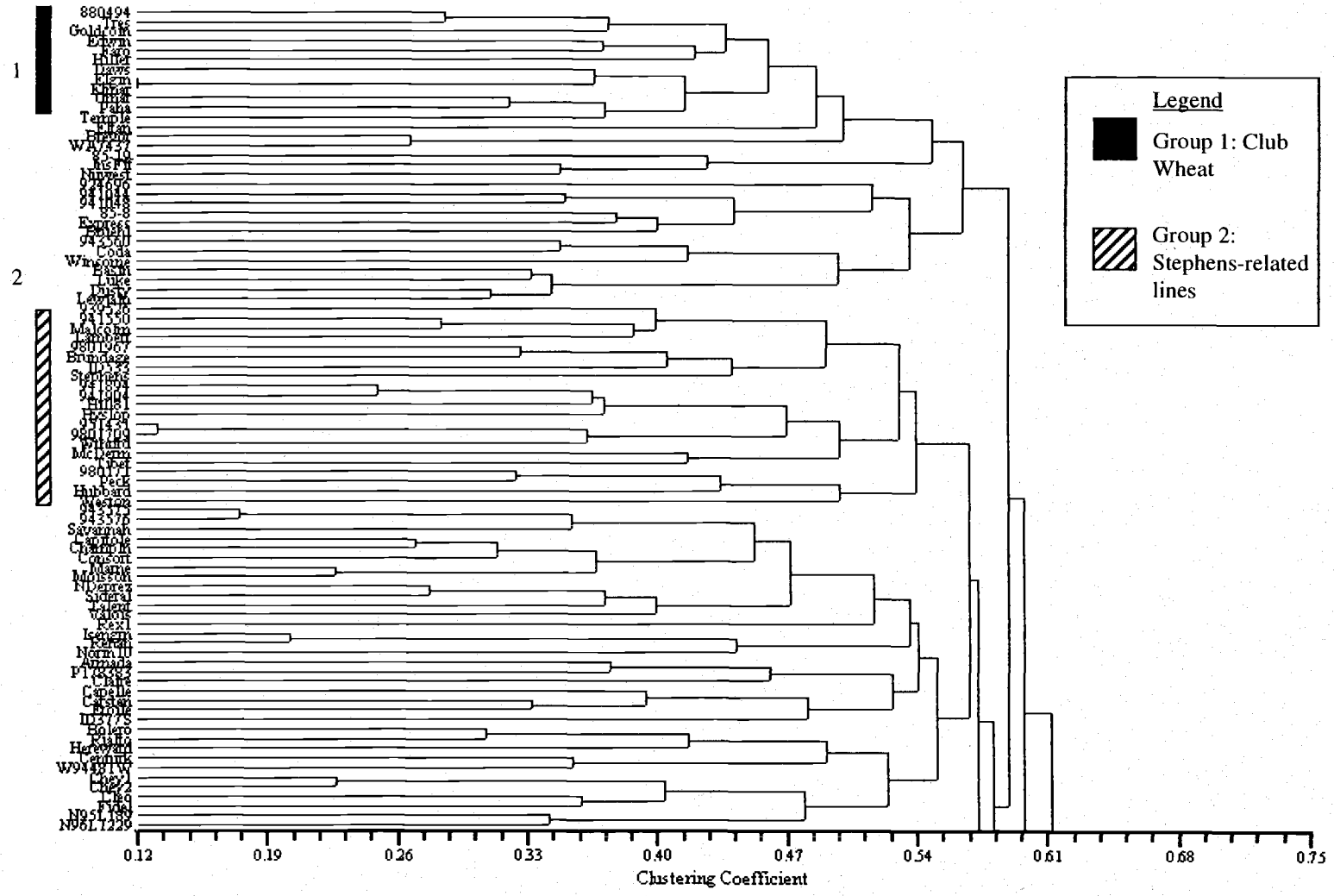

Figure 4b. Genetic relatedness tree from combined nuclear and chloroplast SSR data including club wheat and Stephens-related clusters.

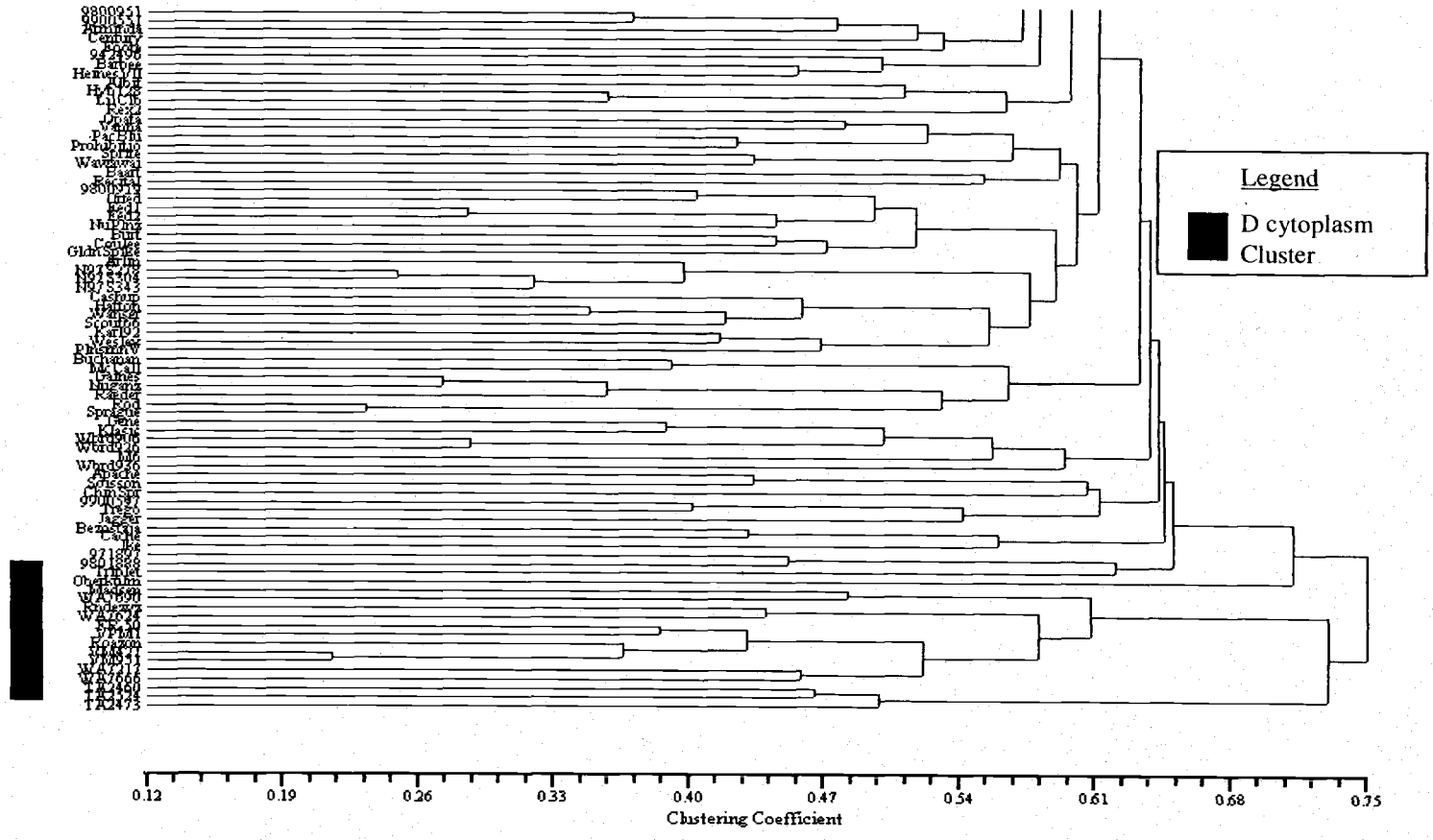

Figure 4c. Genetic relatedness tree from combined nuclear and chloroplast SSR data including the D type cytoplasmic cluster.

The primary clustering commonality of nuclear clustering was geographical region, with secondary clustering based on market class. The chloroplast clustering of cultivars within the B type cytoplasm had no recognizable defining commonality. When the two types of markers are combined, the primary distinction becomes chloroplast type, which appears to distort the nuclear marker clustering. For geographical clustering, nuclear SSRs are more useful in this germplasm pool as based on known and expected cultivar relationships. Chloroplast and nuclear genetic relatedness measurements are not complimentary when the two data sets are combined. For instance, two lines might have the same chloroplast type yet be divergent at the nuclear level. An example of this from this study would be Roazon and Madsen, which cluster together based on chloroplast types, but are in entirely different major clusters based on nuclear data alone. It is therefore more useful to interpret the data separately rather than combining the data analysis.

Historical Lines

After 1949, a series of newer disease resistant lines began to be released in the PNW (Peterson et al. 2001). A second issue that the combined marker information was used to address is the historical genetic diversity of PNW wheat breeding germplasm. This study has examined 25 lines that were significant parents and/or progenitors of cultivars that were grown over a large proportion of the PNW that were released prior to 1950. The genetic diversity estimates from all markers were compared for these lines and a clustering analysis was performed (Figure 5). Proportion of shared alleles

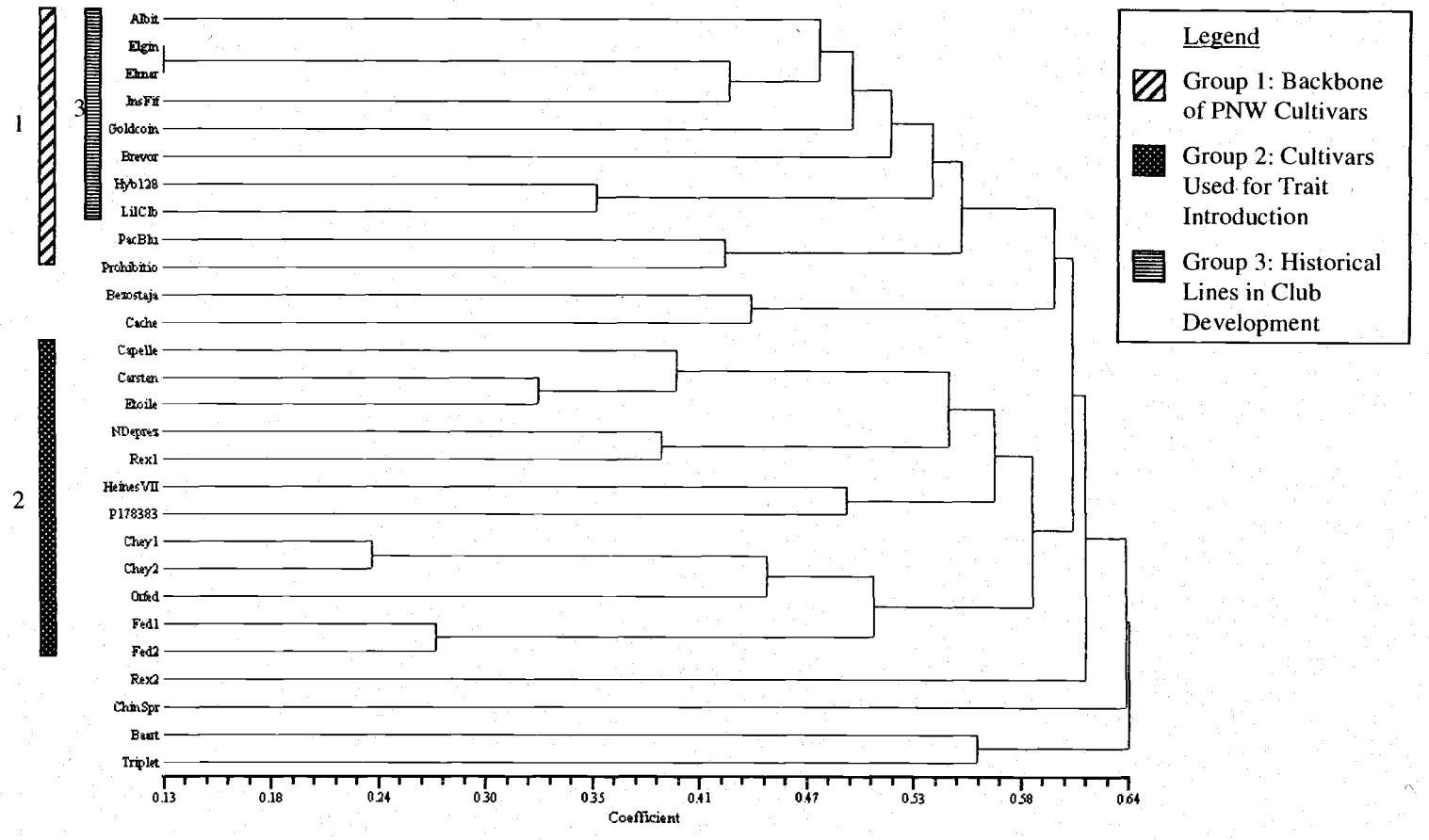

Figure 5. Genetic relatedness tree of Pacific Northwest lines released prior to 1950.

ranged from 87.5% (Elgin and Elmar) to 20.8% (Nord Deprez and Cache). Although most of these lines were expected to be unrelated to one another, none of them share less than twenty percent of their alleles. This could serve as a base threshold of relatedness of genetic material for PNW wheat, regardless of pedigree.

Three of the lines in this study are represented by two different plant introduction (PI) numbers (Federation, Cheyenne, and Rex). The two PIs for Federation and Cheyenne (Appendix 1) were submitted to the National Small Grains Germplasm Repository. These clustered closely, as expected, although they were not quite as similar (0.27 for Federation and 0.236 for Cheyenne) as the parent/offspring duo of Elgin and Elmar. The two PIs for Rex (Appendix 1), however, segregated far from one another, with only 62.9% of alleles shared. When the pedigrees for these two lines were subsequently studied, it was discovered that they are actually two completely different releases. Rex 1 was released in 1962 from a French breeding program, while Rex 2 was released from an Oregonian program in 1933.

The historical lines divide into two specific clusters that were indicative of their roles in PNW wheat breeding programs. Group 1 (coefficient 0.56) consists of the lines that have formed the quality and adaptation backbone of PNW soft white cultivars. Group 2 (coefficient 0.59) consists of lines that have been used in PNW wheat breeding programs for particular trait introgression, such as disease resistance. There are several of these historical lines that cluster together in a stepwise manner (Group 3 in

Figure 5). Many of these are related by pedigree, and all are either club wheat or significant parental lines for club development.

The two lines that shared the fewest alleles were Nord Deprez and Cache. These lines are both hard red winter wheats but completely unrelated based on pedigree, released one year apart (1945 and 1944 respectively). Nord Deprez comes out of a French breeding program and Cache comes from Utah and was derived from the cultivar Turkey. It also corroborates the findings of Roeder et al. (2002) that the primary distinction seems to be based on geographical location.

Heterogeneity

The combined data in this study was also used to address the first objective, to ascertain the usefulness of these markers in this set of germplasm. This study yielded 2.3% heterogeneity for all marker/variety combinations, which is lower than previously reported 4.3% (Roeder et al. 2002). The markers selected for analysis in this study were chosen for ease of scoring, low copy number, which may account for this disparity. For the nuclear markers, 30.1% of the lines had at least one case of heterogeneity observed. This is higher than the 25% found by the European study using several of the same markers. Since this study contains several lines that are breeding lines or unreleased experimental lines, there is most likely higher heterogeneity than that of the pureline European cultivars used in the Roeder et al. (2002) study.

Conclusions

The markers used in this study were selected for high polymorphism and were able to differentiate even closely related or sister lines. All of the nuclear markers used yielded information of varieties and many clusters were related to commonalities in genetic background as based on pedigree comparisons. It is important to have good genome coverage when studying closely related lines as in this study. If the markers are tightly linked, there will be little recombination between them and genetic diversity estimates will be artificially lower than the actual genetic diversity throughout the rest of the genome. Three large wheat germplasm studies using SSRs have been recently published (Manifesto et al. 2000, Roeder et al. 2002, Huang et al. 2002) that used 15, 19, and 24 nuclear markers respectively. This study found the 24 nuclear markers used to be effective and informative, although the presence of some unexplained clustering relationships indicates that more markers might be advisable for future germplasm diversity studies of PNW wheat. The chloroplast markers were effective for differentiating major plasmon types between species and major chloroplast types within species also.

Nuclear markers identified more alleles per locus, with fewer lines on average being defined by the single most common allele. The higher number of alleles allowed more complex groupings than with chloroplast markers. The clusters were mostly found to

have biological basis, with the major distinction being region of adaptation and a secondary distinction based on market class.

Because these markers were able to distinguish between all cultivars included in this study, they could potentially be used in the future for breeding decisions. This information could be useful during gene introgression as a means of tracking the genetic background of the elite cultivar, thus accelerating the selection process toward more rapid regression to the elite cultivar's overall genome. While this method is not guaranteed because of scant genome coverage, it provides another tool that may decrease the amount of time required for breeders to achieve/identify the desired genetic background.

As new material is received into PNW wheat breeding germplasm, fingerprinting any unfamiliar material with this set of markers might help in categorizing genetic similarity or difference with existing germplasm. New material that falls into known clusters could then be integrated into the program as alternatives to others in that cluster. New material that clusters outside of known groups might be useful to introduce new allelic combinations for diversity management.

The chloroplast marker data shows alloplasmic lines within both PNW and European wheat breeding germplasm. The effect of alien cytoplasm on wheat phenotype remains largely unexplored. With the identification of existing cultivars with D type

cytoplasm, these effects could be more readily observable. This set of markers was sufficient to discriminate between B and D type markers, and would be useful in the future to identify which type of cytoplasm other cultivars contain. Additionally, decisions regarding cytoplasmic diversity would be facilitated by this data. Breeders could select the direction of crosses based on desired cytoplasmic type in the offspring. This information could help breeders to take steps that alleviate any concerns regarding cytoplasmic uniformity.

While this study provides valuable information about the nuclear genetic diversity of the cultivars studied, repeating the entire process for several of the nuclear markers would broaden the scope of the study. Over 500 European cultivars have been fingerprinted using several of the same markers (Roeder et al. 2002). Those allele sizes were confirmed and calibrated by repeating each one at least once. With this additional information for these lines, PNW wheat breeding germplasm could be compared and analyzed with this large European database. This would extend the usefulness of this database to wheat breeders in many regions.

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APPENDICES

Appendix 1. List of plant material with abbreviations and seed source.

Label	Accession Name	Identification Number	Seed Source [a]
880494	OR880494		L
924696	ORD924696		L
939526	OR 939526		L
941044	OR941044		L
941048	OR941048		L
941550	OR941550		L
941899	OR941899		L
941904	OR941904		L
942496	OR942496		L
943560	OR943560		L
943575	OR943575		L
943576	OR943576		L
951431	OR951431		L
971897	OR971897		L
980171	OR9801710		L
9800919	OR9800919		L
9801709	OR9801709		L
9801888	OR9801888		L
9801967	OR9801967		L
9900597	OR9900597		L
85-19	OR850513-19		L
85-8	OR850513-8		L
9800951	OR9800924		L
9900551	OR9900553		L
Albit	Albit	CItr 8275	B
Apache	Apache		L

Appendix 1 (Continued).

Label	Accession Name	Identification Number	Seed Source [a]
Arlin	Arlin	PI 564246	E
Armada	Armada		D
Arminda	Arminda		L
Aztec	Aztec		L
Baart	Baart	CItr 1697	B
Barbee	Barbee	CItr 17417	B
Basin	Basin		D
Bezostaja	Bezostaja		D
Bolero	Bolero		L
Brevor	Brevor	CItr 12385	B
Bruehl	Bruehl	PI 606764	B
Brundage	Brundage	PI 599193	H
Buchanan	Buchanan		D
Burt	Burt	CItr 12696	B
C178383	CI 178383		K
Cache	Cache	CItr 11599	B
Capelle	Capelle Deprez		D
Capitole	Capitole		D
Carsten	Carstens V		D
Cashup	Cashup		L
Centurk	Centurk	CItr 15075	B
Century	Century	PI 502912	B
Champln	Champlein		D
Chey1	Cheyenne	CItr 8885	B
Chey2	Cheyenne	PI 192268	B
ChinSpr	Chinese Spring		M

Appendix 1 (Continued).

Label	Accession Name	Identification Number	Seed Source [a]
Claire	Claire		L
Cleo	Cleo		D
Coda	Coda	PI 594372	B
Consort	Consort		L
Coulee	Coulee	CItr 14483	B
Daws	Daws	CItr 17419	B
Dusty	Dusty	PI 486429	B
Edwin	Edwin	PI 606765	B
Elgin	Elgin	CItr 11755	B
Elmar	Elmar	CItr 12392	B
Eltan	Eltan	PI 536994	B
Etoile	Etoile de Choisy		D
Express	Express		D
Faro	Faro	CItr 17590	B
Fed1	Federation	CItr 4734	B
Fed2	Federation	PI 387970	B
Fidel	Fidel		D
Foote	Foote	PI 599663	B
FR-50	FR-50	PI 494183	B
Gaines	Gaines		D
Gene	Gene	PI 560129	B
GldnSpike	Golden Spike	PI 614813	G
Goldcoin	Goldcoin	CItr 4156	B
Gwen	Gwen		D
Harrington	Harrington		J
Hatton	Hatton	CItr 17772	B

Appendix 1 (Continued).

Label	Accession Name	Identification Number	Seed Source [a]
HeinesVII	Heines VII		D
Hereward	Hereward		L
Hill81	Hill 81	CItr 17954	B
Hiller	Hiller	PI 587026	B
Hubbard	Hubbard		L
Hyak	Hyak	PI 511674	B
Hyb128	Hybrid 128	CItr 4512	B
Hyslop	Hyslop		D
ID377S	MTRWA 92-158	PI 564257	B
ID533	92ARS934	PI 590270	B
Ike	Ike	PI 574488	E
Isngrn	Isengrain		L
Jagger	Jagger	PI 593688	B
JnsFif	Jones Fife	CItr 4468	B
Karl92	Karl 92	PI 564245	E
Klasic	Klasic	PI 486139	B
Kmor	Kmor	PI 536995	B
Kold	Kold		D
Lambert	Lambert	PI 583372	B
Lewjain	Lewjain	CItr 17909	B
LilClb	Little Club	CItr 4066	B
Luke	Luke	CItr 14586	B
M6	M6		L
Madsen	Madsen	PI 511673	B
Malcolm	Malcolm	PI 497672	B
Marne	Marne	PI 262226	B

Appendix 1 (Continued).

Label	Accession Name	Identification Number	Seed Source [a]
McCall	McCall	CItr 13842	B
McDerm	McDermid	CItr 14565	B
McVicr	Macvicar	PI 552427	B
Moisson	Moisson	PI 315998	B
Morex	Morex		J
Moro	Moro	CItr 13740	B
N95L189	N95L189		F
N96L1229	N96L1229		L
N97S278	N97S278		F
N97S304	N97S304		F
N97S343	N97S343		F
NDeprez	Nord Deprez		D
Norin10	Norin 10	PI 277364	B
Nuganz	Nugaines	CItr 13968	B
NuPlnz	Nuplains	PI 605741	B
Nuwest	Nuwest	PI 586806	C
Oberkulm	Oberkulmer		L
Omar	Omar	CItr 13072	B
Opata	Opata 85		M
Orfed	Orfed	CItr 11913	B
OWBDom	Oregon Wolfe Barley Dominant		J
OWBRec	Oregon Wolfe Barley Recessive		J
P178383	PI 178383	PI 178383	D
PacBlu	Pacific Bluestem	CItr 4067	B
Paha	Paha	CItr 14485	B
Peck	Peck	CItr 17298	B

Appendix 1 (Continued).

Label	Accession Name	Identification Number	Seed Source [a]
Pllmn101	Pullman 101	CItr 17699	B
PlnsmnV	Plainsman V	PI 591702	B
Prohibition	Prohibition		I
Raeder	Raeder	CItr 17418	B
Recital	Recital		L
Rely	Rely	PI 542401	B
Reman	Reman		L
Rex1	Rex	PI 285088	B
Rex2	Rex	CItr 10065	B
Rialto	Rialto		L
Rndezvz	Rendezvous		D
Roazon	Roazon	PI 422330	B
Rod	Rod	PI 558510	B
Savannah	Savannah		L
Scout66	Scout 66	CItr 13996	B
Sideral	Sideral		L
Soisson	Soissons		L
Sprague	Sprague	CItr 15376	B
Sprite	Sprite		D
Stephens	Stephens	CItr 17596	B
TA2460	TA2460		N
TA2473	TA2473		N
TA2524	TA2524		N
Talent	Talent		D
Temple	Temple	PI 599665	B
Tibet	Tibet		L

Appendix 1 (Continued).

Label	Accession Name	Identification Number	Seed Source [a]
Trego	Trego	PI 612576	B
Tres	Tres	CItr 17917	B
Triplet	Triplet	CItr 5408	B
Tyee	Tyee		D
Valois	Valois		L
Vanna	Vanna		D
VM421	VPM/Moisson, 421		A
VM951	VPM/Moisson, 951		A
VPM1	VPM 1	PI 519303	B
W94481W	W94-481W		L
WA7217	WA 7217	PI 561035	B
WA7437	WA 7437	PI 561033	B
WA7621	WA 7621	PI 566815	B
WA7624	WA 7624	PI 561032	B
WA7625	WA 7625	PI 561031	B
WA7666	WA 7666	PI 561030	B
WA7671	WA 7671	PI 566816	B
WA7690	WA 7690	PI 597665	B
WA7855a	WA 7855		L
WA7855b	WA7855		L
Wanser	Wanser	CItr 13844	B
Wawawai	Wawawai		D
Wbrd906	Westbred 906		D
Wbrd926	Westbred 926		L
Wbrd936	WestBred 936		L
Wesley	Wesley	PI 605742	B

Appendix 1 (Continued).

Label	Accession Name	Identification Number	Seed Source [a]
Weston	Weston	CItr 17727	B
Winsome	Winsome	PI 613177	B
Wthrfrd	Weatherford		L
Yamhill	Yamhill	CItr 14563	B

[a] Seed Source Codes:

A	Dr. R.E. Allan, USDA/ARS, Pullman WA
B	Dr. H. Bockelman, USDA National Small Grains Research Facility
C	Dr. P. Bruckner, Montana State University
D	Dr. X. Chen, USDA Pullman WA
E	Dr. A. Fritz, Kansas State University
F	R. Graybosch, USDA/ARS
G	Dr. D. Hole, Utah State University
H	Dr. E. Souza, University of Idaho
I	J. Bassinette, Oregon State University
J	Dr. P. Hayes, Oregon State University
K	Dr. R. Metzger, Oregon State University
L	Dr. C. J. Peterson, Oregon State University
M	Dr. C. Qualset, University of California, Davis
N	Dr. B. Gill, Kansas State University

Appendix 2. Chloroplast SSR allele sizes for the 174 genotypes studied.

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
880494	183	169	145	100		
924696	108	185	169	145	104	198
939526	108 96	185	167	145	104	198
941044	108	185	169	145	104	198
941048	108 96	183	169	145	104	198
941550	96	185	169	145	104	196
941899	96	185	169	145	104	198
941904	96	185	167	145	104	
942496	108 96	185	169	145	104	198
943560	96	185	167	145	104	196
943575	108	185	167	145	104 100	196
943576	108	185	167	145	104	198
951431	108	185	167	145	104 100	196
971897	108		169	145	104	196
980171	108	183		145	104	196
9800919	108	185	169	145	104	198
9800951	96	185	167	145	104	198
9801709	108	183	167	145	104 100	196
9801888	108 96	185	169	145	104	198
9801967	108	185	169	145	106	198
9900551	96	185	167	145	104	198
9900597	108	185	167	145	104	196
85-19	96	185	169	145	104	198
85-8	108	185	171 169	145	104	198
Albit	108 96	185	169	145	104	196
Apache		167	145	100	196	

Appendix 2 (Continued).

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
Arlin	108		169	145	104	196
Armada	108	185	167	145	104	196
Arminda	185	167	145	104	196	
Aztec	108		169	145	104	
Baart	108	185	169	145	104	198
Barbee	108	185 171	169	145	104	198
Basin	96	185	169	145	106	198
Bezostaja	96	185	169	145	104 100	196
Bolero		185	167	145		198
Brevor	108 96	185	169	145	104	196
Bruehl		185 171	169	145	104 106	198
Brundage	108	185	169	145	106	198
Buchanan	96	185 171	167	145	104 106	196
Burt	108	185	169	145	104	198
C178383			145		198	
Cache		169	145	100	196	
Capelle	108	185 171	169	145	104	196
Capitole	108	185	167	145	104	198
Carsten	108	185 171	167	145	104	196
Cashup				145	100	196
Centurk	108	185		145	104	198
Century	108	185	167	145	104	198
Champln	108	185	167	145	104	198
Chey1		169	145	104	196	
Chey2		185	169	145	104	198
ChinSpr	108	185	167	145	104 100	196

Appendix 2 (Continued.)

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
Claire			145	100	196	
Cleo	108	185	169	145	104	196
Coda	96	185 171	169	145	104	196
Consort	108	185	167	145	104	196
Coulee	96	185	169	145	104 100	198
Daws	96	185	169	145	104	198
Dusty	96	183	169	145	104	196
Edwin	96	183	167	145	104	196
Elgin	96	185	169	145	104 100	198
Elmar	96	185	169	145	104 100	198
Eltan	96	185	169	145	104 100	196
Etoile		185 171	167	145	100 106	196
Express	108	185 171	169	145	104	198
Faro	96	183	169	145	104 100	198
Fed1	108	185	169	145	104 100	198
Fed2	108	185	173	145	104 100	196
Fidel		185	169	145	104 100	196
Foote	108	183	167	145	104	198
FR-50	108	193	163	145	102	196
Gaines	108	185	169	145	104 100	198
Gene	108	185 171	167	145	104	198
GldnSpike	108	185	169		104 100	198
Goldcoin		183	169	145	104	196
Gwen	108	187	173	143	100	198
Harrington	108	187	171	143	100	198
Hatton		185	169	145	104	196

Appendix 2 (Continued).

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
HeinesVII	108	185	169	145	104 100	198
Hereward	108		169	145	104 100	198
Hill81	108	185	167	145	104	198
Hiller	96	183	169	145	104 100	196
Hubbard	108	185	167	145	104	196
Hyak	108 96		169	145	104 100	198
Hyb128	96	185	167	145	106	198
Hyslop	96	185	167	145	104 100	198
ID377S	108	185	167	145	100 106	196
ID533		185	167	145	106	198
Ike		185	169	145	104 100	196
Isngrn	108	185	167	145	104	198
Jagger		183	167	145	104	198
JnsFif		185	169	145	104	198
Karl92		185	169	145	104	196
Klasic		185	167	145	104	198
Kmor	96			145		196
Kold	108	187	173	143		198
Lambert		185	169	145	104	196
Lewjain	96	185	169	145	104 100	198
LilClb		185	169	145	106	196
Luke	96	185	167	145	104	196
M6	108	185	169	145	106 104 100	196
Madsen	108	193	163	143	102	198
Malcolm		183	169	145	104 100	196
Marne	108	185	167	145	104	198

Appendix 2 (Continued).

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
McCall	108 96	185	167	145	104	196
McDerm	96	185	167	145	104	196
McVicr		185	169	145	104	196
Moisson	108	185 171	167	145	104	198
Morex	108	187 173	173	143	100	198
Moro	108		169	145	100	
N95L189	108	183	169	145	104 100	196
N96L1229	108	185	169	145	104	198
N97S278	108	185	169	145	104 100	196
N97S304		183	169	145	104	198
N97S343	108	183	169	145	104 100	198
NDeprez	185	167	145	104	198	
Norin10	108 96	185	169	145	106	198
Nuganz	185 171	167	145	104	198	
NuPlnz	108	185	169	145	104	198
Nuwest	185	169	145	104	198	
Oberkulm	108	185	167	145	102	198
Omar		169	145	100	196	
Opata	108	185 171	169	145	104 100	
Orfed	108	185	169	145	104	198
OWBDom			171	143		
OWBRec		173	143	100	196	
P178383	108	185	169	145	104	196
PacBlu	108	185	169	145	104 100	196
Paha	108 96	185	167	145	104	198
Peck		169	145	100		

Appendix 2 (Continued).

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
Pllmn101	96	185	169	145	104	196
PlnsmnV		183	169	145	104	196
Prohibition		185	169	145	104 100	196
Raeder			145		196	
Recital	110	185	169	145	104 100	196
Rely		185	167		104	198
Reman	108	185	167	145	104	198
Rex1	108	185 171	167	145	104	198
Rex2		185	167	145	104	196
Rialto		183	167	145	104	196
Rndezvz	108	193	163	143	102	196
Roazon	108	193	163	143	104	198
Rod		185	169	145	104 100	198
Savannah	108	183	167	145	104	198
Scout66			169	145	100	196
Sideral	108	185	167	145	104 100	198
Soisson	96	183	167	145	104 100	196
Sprague	108		169	145	104 100	196
Sprite		183	169	145	104	196
Stephens	108	185 171	169	145	106	196
TA2460	108	193	165	145	100	196
TA2473	108	195	161	147	102	196
TA2524	108	191	163	145	100	196
Talent	108	185	167	145	104 100	196
Temple	96	185	169	145	104	198
Tibet		185	167	145	104	196

Appendix 2 (Continued).

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
Trego			167	145	104	196
Tres	96	183	169	145	106	196
Triplet		183	169	145	104	198
Tyee		183	169	145	104	198
Valois	108	185	167	145	104	198
Vanna		183	169	145	104 100	196
VM421	108	191	163	143	102	196
VM951	108	191	163	143	102	196
VPM1	108	193	163	143	102	194
W94481W		185	169	145	104	198
WA7217	108 96	193 177	163	143	102	198
WA7437		185	169	145	104	196
WA7621				143		
WA7624	108	193	163	143	102	196
WA7625	108		163	143		
WA7666	108	191	163	143	102	196
WA7671			163		102	196
WA7690			163	143	100	194
WA7855a	96	183	169	145	104	196
WA7855b	106	187 173	171	143	100	198
Wanser			169	145	100	196
Wawawai			169	145	104 100	196
Wbrd906				145	100	196
Wbrd926	108		169	145	104 100	196
Wbrd936	108	183	167	145	102	196

Appendix 2 (Continued).

Genotype	wct1	wct10	wct11	wct12	wct13	wct14
Wesley	108	185	169	145	104 100	196
Weston	96		169	145	104 100	196
Winsome	96	185	169	145	104	198
Wthrfrd	96		169	145	104 100	196
Yamhill		185 171	169	145	106	196

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
880494		144	196		195	197	184
924696	193	144	196	150	195	197	186
939526	183	142	196	148	195		
941044	185	144	196	150	195	197	186
941048	185	144	196	150	195	197	186
941550	183	142	196	150	195		186
941899	183	142	196	150	195	197	184
941904	183	142	196	150	195	197	186
942496	187	144		150	195	197	184
943560	183	142	196	150	195	197	186
943575	189	142	196	150	195	197	186
943576	189	142	196	148	195	197	186
951431	183	142	196	150	195	197	186
971897	147	142	196	148	195	197	184
980171	183	142	196	148	195	197	
9800919	191	144	196	150	195	197	186
9800951		142	196	150	195	197	
9801709	183	144	196	150	195	197	186
9801888	139	142	196	150	195	197	184
9801967	183	142	196	150	195	197	186
9900551	191	142	196	148	195	197	184
9900597	181	142	196	148		197	
85-19	183	144	196	148	195	197	186
85-8	183	142	196	150	195	197	186
Albit	183	142	196	148	195	197	186
Apache	179	142	196	148	195	197	

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
Arlin	187		196	148	195	197	186
Armada	189	142	196	150	195	197	184
Arminda	181	142	196	150	195	197	184
Aztec					195	197	
Baart	181	142	196	150	195	197	184
Barbee	191	142	194 196	150	195	197	186
Basin	193		196	148	195	197	186
Bezostaja	189		196		195	197	186
Bolero		142	196	148	195	197	
Brevor	179	142	194	148	195	197	186
Bruehl		142	196	150	195	197	184
Brundage			196	148	195	197	
Buchanan	183		196	148	195	197	186
Burt	179	144	196	150	195	197	186
C178383				195	195		
Cache		194	148	195	197	186	
Capelle	187	142	196	148	195	197	186
Capitole	187	142	196	148	195	197	186
Carsten	187	142	196	150	195	197	186
Cashup	189		196		195	197	184
Centurk	185	142	196	148	195	197	184
Century	185	144	196	148	195	197	186
Champln	187	142	194 196	148	195	197	186
Chey1	183	142		148	195	197	186
Chey2		142	196	150	195	197	
ChinSpr	181 185	142	196	148	195	197	184

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
Claire	189		196	148	195	197	184
Cleo		142	194 196	150	195	197	186
Coda	189	144	196	150	195	197	186
Consort	189	142	196	148	195	197	186
Coulee	183	144	196	150	195	197	186
Daws	187	142	196	148	195	197	186
Dusty	191	142	196	148	195	197	184
Edwin	179	142	196	150	195	197	186
Elgin	177	142	196	150	195	197	186
Elmar	177	142	196	148	195	197	186
Eltan	191	142	196	148	195	197	186
Etoile		142	196	150	195	197	186
Express		144	196	150	195	197	186
Faro	179	144	196	150	195	197	186
Fed1	141	142	196	150	195	197	186
Fed2		142	196	150	195	197	186
Fidel	187	142	196	150	195	197	186
Foote	185	142	196	150	195	197	186
FR-50	183	140	194	148	195	195	186
Gaines	183	142	196	148	195		184
Gene	189		196	150	195	197	186
GldnSpike		142	196	150	197	197	
Goldcoin	181	144	196	148	195	197	186
Gwen	181	142	194 196	150	197		192
Harrington	181	142	194	148	197	197 179	184

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
Hatton	183	142	196	148		195	186
HeinesVII		142		150	195	197	184
Hereward189		196	148	195	197	184	
Hill81	183	142	194 196	150	195	197	
Hiller	179		196	146	195	197	186
Hubbard	181	142	196	148	195	197	184
Hyak	191	142	196	148	195		186
Hyb128	181	142	194 196	150	195	197	186
Hyslop	183	142	196	150	195	197	184
ID377S	141	142	196	150	195	197	186
ID533	191	142	196	148	195	197	
Ike			196	148	195	197	
Isngrn	185	142	196	150	195	197	186
Jagger	183	142	196	148	195	197	190
JnsFif	169	142	196	148	195	197	184
Karl92		142	196	148	195	197	188
Klasic	191	142	196	150	195	197	186
Kmor			196		195		184
Kold	179	142	194 196	150	197	179	192
Lambert	183	142	196	148	195	197	186
Lewjain	193	142	196	150	195	197	186
LilClb		142	194 196	148	195	197	
Luke	193	142	196	150	195	197	184
M6		142	196	148 150	195	197	184186
Madsen	181	142	194	148	195	195	188

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
Malcolm	183	142	196	148	195	197	188
Marne	189		196	150	195	197	186
McDerm	189	142	194 196	150	195	197	184
McVicr	183	142	196	150	195	197	186
Moisson	139		196	150	195	197	186
Morex	179	142	194	148	197	197	190
Moro				148	195	197	
N95L189	181	142	196	148	195	197	186
N96L1229	179	142	196	150	195	197	186
N97S278	185	142	196	148	195	197	184
N97S304		142	196	150	195	197	186
N97S343	185	144	196	150	195	197	186
NDeprez	187		196	150	195	197	
Norin10	187	142	196	150	195	197	
Nuganz	183	142	196	150			186
NuPlnz		142	196	148	195	197	
Nuwest		142	196	150	195	197	
Oberkulm	177	142	194 196	150	197	197	184
Omar	179		196	148	195	197	
Opata	181	142	194 196	148	195	197	184
Orfed	183	142	196	150	195	197	186
OWBDom		142	194	150	197	197	
OWBRec		142	194		197		
P178383	191	142	196	150	195	197	184

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
PacBlu	181	142	196	150	195	197	186
Paha	179	142	196	150	195	197	186
Peck	183		196	148	195	197	
Pllmn101	181	142	196	148	195	197	184
PlnsmnV	191	142	196	148	195	197	184
Prohibition	181		196	148	195	197	186
Raeder					195	197	
Recital	187	142	196	150	195	197	186
Rely		144	196	150	195	197	186
Reman	179	142		150	195	197	186
Rex1	181	144	196	150	195	197	
Rex2	141	142	196	150	195	197	184
Rialto	191	142	196	148	195	197	186
Rndezvz	187	140	196	150	195	195	184
Roazon	179	142	194	148	195	197	186
Rod	183	142	196	148	195	197	184
Savannah	189	142	196	150	195	197	186
Scout66			196	148	195	197	184
Sideral	179	142	196	150	195	197	186
Soisson	181	142		148	195	197	184
Sprague	183	142	196	150	195	197	
Sprite	185		196	150	195	197	184
Stephens	191	142	194 196	150	195	197	186
TA2460	179	142	194	148	195	195	182

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
TA2473	175		194	146	195		182
TA2524	181		194	146	195	195	182
Talent	187	142	196	150	195	197	186
Temple		144	196	150	195	197	
Tibet	189	142	196	150	197	197	186
Trego	187	142	196		195	197	
Tres	181	144	196	150	195	197	184
Triplet		142	196	148	195	197	184
Tyee		142	196	150	195	197	188
Valois	187		196	148	195	197	184
Vanna	187	142	196	150	195	197	186
VM421	187		194	148	195	193	186
VM951	189		194	148	195	195	186
VPM1	189	140	194	148	195	195	190
W94481W	185	142	196	150	195	197	182
WA7217	191		194 196	148	195	195	186
WA7437		144	194	148	195	197	
WA7621					195	195	
WA7624	187		194	148	195	195	186
WA7625					195		186
WA7666	183		194	148	195		186
WA7671			194	148	195	195	
WA7690			194		195	195	192
WA7855a	181		196	148	195	197	
WA7855b		142	194	150	199	179	190

Appendix 2 (Continued).

Genotype	wct15	wct17	wct18	wct19	wct22	wct4	wct6
Wanser			196		195	197	186
Wawawai			196	150	195	197	182
Wbrd906		142		150	195	197	
Wbrd926	181	142	196	148	195	197	186
Wbrd936	179	142	196	150	195	197	184
Wesley	191	142	196	148	195	197	186
Weston	183		196		195	197	184
Winsome	187	142	196	150	195	197	186
Wthrfrd	183		196	150	195	197	186
Yamhill		142	196	150	195	197	186

Appendix 2 (Continued).

Genotype	wct8	wct9
880494	144	120
924696		120
939526	144	120
941044	144	118
941048	144	120
941550	146	120
941899	144	120
941904	144	120
942496	146	120
943560	144	120
943575	144	118
943576	144	118
951431	146	120
971897	146	118
980171	144 134	120
9800919	146	120
9800951	146	120
9801709	144	120
9801888	144	118
9801967		118
9900551	144	118
9900597	144	120
85-19	144	120
85-8	144	120
Albit	144	120
Apache	144	

Appendix 2 (Continued).

Genotype	wct8	wct9
Arlin	146	118
Armada	144	120
Arminda	144	120
Aztec		
Baart	146	120
Barbee	146	120
Basin	144	118
Bezostaja	146	120
Bolero	146	120
Brevor	144	118
Bruehl	144	116
Brundage	144	120
Buchanan	146	120
Burt	146	120
C178383		
Cache		120
Capelle	144	120
Capitole	146	120
Carsten	144	120
Cashup	144	116
Centurk	144	118
Century	144	118
Champln	144	120
Chey1	144	120
Chey2	144	
ChinSpr	146	116 120

Appendix 2 (Continued).

Genotype	wct8	wct9
Claire	146	120
Cleo	144	120
Coda	144	120
Consort	144	120
Coulee	146	120
Daws	144	120
Dusty	144	120
Edwin	144	120
Elgin	144	120
Elmar	144	120
Eltan	146	120
Etoile	146	
Express	144	
Faro	144	120
Fed1	146	120
Fed2	144	120
Fidel	145	120
Foote	144	120
FR-50	142	116
Gaines	146	120
Gene	144	120
GldnSpike	144	
Goldcoin	144	120
Gwen	138	118
Harrington	146 138	118
Hatton	144	116

Appendix 2 (Continued).

Genotype	wct8	wct9
HeinesVII	146	120
Hereward146	120	
Hill81	146	120
Hiller	144	120
Hubbard	144	120
Hyak	146	
Hyb128	144	120
Hyslop	146	120
ID377S	144	120
ID533	144	120
Ike	144	120
Isngrn	144	118
Jagger	146	
JnsFif	144	120
Karl92	144	
Klasic	144	120
Kmor	146	
Kold	138	118
Lambert	144	120
Lewjain	144	120
LilClb	144	120
Luke	144	120
M6	144	120
Madsen	142	116
Malcolm	144	120
Marne	144	120

Appendix 2 (Continued). Chloroplast SSR allele sizes for the 174 genotypes studied.

Genotype	wct8	wct9
McCall	146	120
McDerm	144	120
McVicr	144	120
Moisson	144	120
Morex	138	118
Moro		
N95L189	144	120
N96L1229	146	120
N97S278	146	120
N97S304	144	120
N97S343	146	120
NDeprez	144	120
Norin10	144	118
Nuganz	144	118
NuPlnz	146	120
Nuwest	144	
Oberkulm	146	120
Omar	146	
Opata	144	120
Orfed	146	118
OWBDom	138	118
OWBRec		118
P178383	146	120
PacBlu	146	120
Paha	146	120
Peck	144	120

Appendix 2 (Continued).

Genotype	wct8	wct9
Pllmn101	146	
PlnsmnV	146	
Prohibition	146	120
Raeder	146	
Recital		120
Rely	144	
Reman	144	118
Rex1	144	120
Rex2	144	
Rialto	146	120
Rndezvz	144	116
Roazon	142	116
Rod	144	120
Savannah	144	120
Scout66	146	
Sideral	146	120
Soisson	144	120
Sprague	144	116
Sprite	146	
Stephens	144	120
TA2460	144	120
TA2473	144	120
TA2524	144	120
Talent	144	116
Temple	144	120
Tibet	144	120

Appendix 2 (Continued).

Genotype	wct8	wct9
Trego	146	116
Tres	144	120
Triplet	144	116
Tyee	144	116
Valois	146	120
Vanna	144	120
VM421	142	116
VM951	142	116
VPM1	142	116
W94481W	146	120
WA7217	142	116
WA7437	144	
WA7621		116
WA7624	144	116
WA7625	142	116
WA7666	144	116
WA7671	142	
WA7690	144	
WA7855a	144	116
WA7855b	138	118
Wanser		120
Wawawai	146	
Wbrd906	144	
Wbrd926	146	120
Wbrd936	146	118

Appendix 2 (Continued).

Genotype	wct8	wct9
Wesley	146	120
Weston	144	120
Winsome	144	120
Wthrfrd	144	120
Yamhill	144	

Appendix 3. Nuclear SSR allele sizes for the 171 genotypes assayed.

Genotypes	gwm135	gwm148	gwm155	gwm160	gwm169	gwm18
880494	148	160	144	179	198	190
924696	142	130	179		192	
939526	136 148	160	144	179		
941044	128	140	146	179	190	190
941048	136	144	146	179	190 200	190
941550	128 148	144	146	179	196	192
941894	136	160	146	181	200	190
941904	148	160	146	179	200	190
942496	136 148	164	148	179	198	192
943560	148	160	144	179	200	186
943575	136 148	144	146	179	198	190
943576		160	146	179	196	192
951431	148	144	144	179	200	190
971897		140	130	179		194
980171	148		146	181	194	192
9800919		144	146	175	200	192
9800951		162	126	179	196	192
9801709	148	144	144	181	200	190
9801888		140	130		188	188
9801967	136	162	146	173	200	192
9900551	148	162	146	181		192
9900597	148	162		179	196	190
85-19	148	144	150	173	196	190
85-8	148	144	146	179	190	190
Albit	136	160	146	175	190	190
Apache		144	146	175		192

Appendix 3 (Continued).

Genotypes	gwm135	gwm148	gwm155	gwm160	gwm169	gwm18
Arlin	136	140	142	179		190
Armada	136	144	146	179	194	190
Arminda	136		148	185		192
Aztec	146	144	146	173	194	190
Baart	148	140	148	175	186	196
Barbee	136	140	144	173	184	192
Basin	148	162	146	179	188	
Bezostaja	136	140	142	177		188
Bolero	148		146	179	106	190
Brevor	148	162	146	173		190
Bruehl	148	160			190	190
Brundage			146	173		192
Buchanan	136	140	144	187	196	192
Burt		146	142	173	198	190
C178383		142	146	173		190
Cache				177		188
Capelle	136	146		179	200	190
Capitole	136	160		179	200	190
Carsten	136	140	146	179	200	192
Cashup		160		179		190
Centurk		140	150			190
Century		162	152	179		192
Champln	136	144	146	179	200	190
Chey1			144	179		192
Chey2			144	179		118
ChinSpr	138	160	144	173	194	188

Appendix 3 (Continued).

Genotypes	gwm135	gwm148	gwm155	gwm160	gwm169	gwm18
Claire	136	144		179	192	192
Cleo	136	144		185	196	192
Coda	148	160			200	190
Consort	136	144	146	173		190
Coulee		162	142	173	196	190
Daws	136	160	146	179	196	192
Dusty	148	162	146	179		190
Edwin		160		179	184	192
Elgin		160	146	173	188	190
Elmar		160			188	190
Eltan	148	160		173	184 196	190
Etoile	136		146	181		192
Express			146	179	188	190
Faro	148	160	146	173	192	190
Fed1		162	142	187	186	196
Fed2		162	142	185		196
Fidel	136	144	144	179		192
Foote	148	162		179	198	188
FR-50	136	144	144	179	188	192
Gaines		140	146	185	198	192
Gene		164	146		192	190
GldnSpike	136	144	146			190
GoldCn	148	160	146	173		
Hatton			146	179		
Heines	136	144	144	189	188	192
Herewrd	136	144	144	179	188	190

Appendix 3 (Continued).

Genotypes	gwm135	gwm148	gwm155	gwm160	gwm169	gwm18
Hill81	148	160	146	179	200	190
Hiller	148	160	146	181	190	190
Hubbard	148	162	146	179	198	192
Hyb128		144	146	185	196	190
Hyslop		160	146	179		192
ID377S	136	142	144	181	188	192
ID533		162	144	179		190
Ike	136		150	181		194
Isengrn	136	140	144	179	186	192
Jagger		144	148	179		188
JnsFif		162	144	173		190
Karl92	140	140	142		196	192
Klasic		144	144	185		192
Kmor				179		192
Lambert	134 148			179	198	192
Lewjain	148	162		179	184	192
LilClb	148	144	146	185		190
Luke		162		179	184	192
M6	138	164		187	192	182
Madsen	148		144	179	200	192
Malcolm	136 148	162	146	179		192
Marne	136	144	144	181	200	190
McCall	136 148	144	146	179	196	192
McDerm		162		181	198	
Moisson	136		146	181	200	190
Moro	128	162	146	175	196	190

Appendix 3 (Continued).

Genotypes	gwm135	gwm148	gwm155	gwm160	gwm169	gwm18
N95L189	136	162	150	175	190	192
N96L1229	136	162	150	177	186	192
N97S278	136	140	146	179	188	196
N97S304	136	140	146	179		196
N97S343	140	140	146	179	196	196
NDeprez			148	179		198
Norin10		160	126	173	186	192
Nuganz	136 148	140	146	187	198	
NuPlnz	140	162	146			184
Nuwest	136		150	173		188
Oberkulm		158		175	194	192
Omar		160		179		192
Opata	148	162	146	173	186	190
Orfed		140	142	185	196	192
P178383	136	142	146	179		192
PacBlu	136	160	146	175		190
Paha	148	160	126	185		188
Peck		140	146	185		192
PlnsmnV		144	150	177		192
Prohibit	148	160	146	185		190
Raeder			146	185		190
Recital	146	144	146	175	190	188
Reman	136	140	144	179	186	188
Rex1	136	144	144	179	196	182
Rex2	136	162	142	173	196	190
Rialto	136 148	144	144	179		192

Appendix 3 (Continued).

Genotypes	gwm135	gwm148	gwm155	gwm160	gwm169	gwm18
Rndezvz		144	144	173		188
Roazon	136	160	148	179	200	190
Rod	132	140	146	187		192
Savannah	148	144	146	181	190	192
Scout66		144				192
Sideral		144	148	179	192	190
Soisson	134	140	146	179	190	192
Sprague	132		146	187	196	192
Sprite		140	146	179		190
Stephens	136	140		179	196	190
TA2460	148	144		181	188	190
TA2473	148	144				186
TA2524	148	144	146			
Talent	136	160	146	179	198	190
Temple	148	160	146	179		192
Tibet	146	144	150	179	190	190
Trego	136	162	150	179		188
Tres	148		144	179	190	190
Triplet	148		144	173		192
Valois	136	144	144	179	198	190
Vanna		140				190
VM421	136	160	144	181	200	190
VM951	136	160	144	179	188 200	190
VPM1	136	144	144	179		190
W94481W		162	150	179		190

Appendix 3 (Continued).

Genotypes	gwm135	gwm148	gwm155	gwm160	gwm169	gwm18
WA7217	136	140	146	173	200	192
WA7437	136 148	160		173	188	190
WA7621	148	160	146	173		190
WA7624	148	140	146	173	196	190
WA7666	136	162	146	173	198	190
WA7671	148		146	181		190
WA7690		162	144	181		192
Wanser		162	146	179		192
Wawawai		160	146	179		190
Wbrd906		164	144	175		190
Wbrd926		164	148	185		190
Wbrd936	136 148	164	128	179	190	192
Wesley		144	142	177		192
Weston	128 152	164	146	179	184	192
Winsome	148	160	148	179		192
Wthrfrd	148	160	144	181		192

Appendix 3 (Continued).

Genotypes	gwm190	gwm194	gwm234	gwm261	gwm282	gwm3
880494	208		225	175	225	85
924696	210	129	223	165	231	
939526	212	127	225	175	199	79 85
941044	206	133	245	175	231	79
941048	206	129	235	175	187	79
941550	212	127	241	175	197	79
941894	202	133	225	175	193	85
941904	202	129	225	175	193	85
942496	206	131	223	175	195 219	79
943560	214	129	245	193	193	79
943575	202	135	241	175	193	77
943576	202	135	225	175	193	77
951431	202	127	225	175	187	85
971897	206	117	237	175	183	79
980171	210	131		175	195	
9800919	212	131		165	193	85
9800951			225	165	187	79
9801709	202	129		175	195	85
9801888	212	117	223	175	207	
9801967	210	131	237	175	223	77
9900551	212	131	225	165	193	79
9900597		131	237	197	263	83
85-19	200	135	245	163	199	79
85-8	210	129	237	163	231	79
Albit	212	129	239	175	231	77
Apache	212	135	239	175	199	79

Appendix 3 (Continued).

Genotypes	gwm190	gwm194	gwm234	gwm261	gwm282	gwm3
Arlin	206	133	235	163	231	85
Armada	212	129	235	175	223	79
Arminda	208	129		165		79
Aztec	212	129		175		79
Baart	212	131	225	173	195	85
Barbee	210	133	225	175	257	79
Basin	210	133	243	175	193	79
Bezostaja	212	131	235	175 193	225	79
Bolero	212	129		175		
Brevor	210	133		173	169 231	79
Bruehl		127	233		193	79
Brundage	202 210			175		77
Buchanan	214	135	245	175		83
Burt	214	129	225	165	181	81
C178383		115	241		195	79
Cache	202 212			165		83
Capelle	212	133	237	175	197	79
Capitole	214	133	239	193	193	77
Carsten	212	133	241	175	269	79
Cashup	212	135	243	173	197	
Centurk	210	127	235	197	263	83
Century	210	131	245	165	231	79
Champln	212 214	131	241	175	207	77
Chey1	210	131		211		83
Chey2	210			211		
ChinSpr	212	129	237	193	217	79

Appendix 3 (Continued).

Genotypes	gwm190	gwm194	gwm234	gwm261	gwm282	gwm3
Claire	214	137	241	165	197	79
Cleo	202	129	241	175	185	77
Coda		129	237		185	79
Consort	214	135	241	163	187	77
Coulee	214	135	245	175	269	85
Daws	210	133	225	175	197	85
Dusty	210	135	243	175	193	79
Edwin		135	225	175	187	85
Elgin		133	225	175	185	85
Elmar		133	237	175	185	85
Eltan	224	135	235	175	193	85
Etoile	212			193		79
Express	206			165		79
Faro	208	135	225	175	183	85
Fed1	218	131	235	163	263	81
Fed2	218	131	245	165	167	85
Fidel	210	133	241	175	259	85
Foote	206	131	235	165	193	85
FR-50	214	131	249	175	187	85
Gaines	210	133	245	175	269	85
Gene	200	133	241	163	195	77
GldnSpike	210		245	165	269	85
GoldCn	218	135	225	175	185	85
Hatton	212	135		175		85
Heines	202	131	225	175	223	79
Herewrd	212	125	245	165	195	77

Appendix 3 (Continued).

Genotypes	gwm190	gwm194	gwm234	gwm261	gwm282	gwm3
Hill81	210	129	225	175	193	85
Hiller	210 216 218	135	235	175	231	85
Hubbard	210	127	225	173	191	85
Hyb128	218	129	237	175	197	85
Hyslop	212	133	225	175	193	85
ID377S	212	129	225	175	193	79
ID533	210	135		175		
Ike	214		237	211	201	83
Isengrn	212	135	235	175	195	79
Jagger	206	131	237	163	185	
JnsFif	216	129	237	175	237	
Karl92	206	133	235	165	263	85
Klasic	204	133		163		85
Kmor	210	135		175		79
Lambert	218	133	225	175	207	77
Lewjain	208	115	243	175	193	79
LilClb	212	131	237	175	195	77
Luke	210	113	243	175	193	79
M6	182 196			195		71
Madsen	202	129	237	175	193	85
Malcolm	212	131	225	175	199	79
Marne	214	131	235	175	187	77
McCall	214	135	245	175	231	85
McDerm	202	133	227	175	199	85
Moisson	214	131				79
Moro	206	135		173	185	79

Appendix 3 (Continued).

Genotypes	gwm190	gwm194	gwm234	gwm261	gwm282	gwm3
N95L189	212	131	241	197	231	85
N96L1229	212	131	241	197	231	
N97S278	210	133	235	197	231	85
N97S304	210	133		165 197	231	85
N97S343	206	131	235	165	231	85
Ndeprez	212	131		175		77
Norin10	214	131	235	193	193	79
Nuganz	210	133	245	175	269	85
NuPlnz	210	131	245	197	227	85
Nuwest	208			163		83
Oberkulm	216	131	225	165	257	83
Omar	216	133	237	175	185	85
Opata	214	133	245	165	263	
Orfed	208	131	235	165	233	81
P178383	208	115	243	175	195	79
PacBlu	218	133	235	163	167	79
Paha	216	135	237	175	185	85
Peck	210	137	245	175	271	85
PlnsmnV	212	131	237	165	195	85
Prohibit	210	131	241	175	235	79
Raeder	210 212			175		85
Recital	204	131	241	173	195	85
Reman	212	133	235	175	195	79
Rex1	212	131	239	165	199	79
Rex2	212	129	243	175	209	85
Rialto	212	129	237	175	271	77

Appendix 3 (Continued).

Genotypes	gwm190	gwm194	gwm234	gwm261	gwm282	gwm3
Rndezvz	202	135	241	175	187	77
Roazon	194	133	239	193	187	77
Rod	208	133	245	175	209	79
Savannah	202	135	241	175	187	77
Scout66	212	135	235	209	201	79
Sideral	210	129	241	175	221	
Soisson	212	131	235	175	195	79
Sprague	207	137	245	175	211	79
Sprite	214	131	235	163	221	79
Stephens	210	127	225	175	195	77
TA2460	214	129	237	189	231	67
TA2473	252	129	235	195	231	73
TA2524	210	105	239	207	231	79
Talent	212	131	241	175	219	77
Temple	216	131	237	175	231	85
Tibet	212	133	245	175	195	85
Trego	212	131	243	197	225	83
Tres	210	135	237	175	231	85
Triplet	212	133	239	227	187	77
Valois	200	131	239	163	199	77
Vanna	212	133	225	165	227	79
VM421	214	129	225	193	187	77
VM951	214		239	175	187	77
VPM1	214	131		175	263	77
W94481W	212	129	237	163	263	83
WA7217	210	135	225	175	187	85

Appendix 3 (Continued).

Genotypes	gwm190	gwm194	gwm234	gwm261	gwm282	gwm3
WA7437	218	133		173	185	85
WA7621	210	131	237	175		85
WA7624	212	135	225	175	207	77
WA7666	214	135	245	175	193	85
WA7671	202			175		85
WA7690	214	129	239	175	193	85
Wanser	214	135	243	173	267	85
Wawawai	214	131	239	165	221	85
Wbrd906	206	129	229	163	193	85
Wbrd926	206	129	229	163	195	85
Wbrd936	206	133	227	165	195	79
Wesley	214	133	241	193	231	85
Weston	210	131	235	173	231	85
Winsome	212	135	231	193	221	79
Wthrfrd	202 212 218	129	225 239	175	187	85

Appendix 3 (Continued).

Genotypes	gwm334	gwm389	gwm437	gwm458	gwm46	gwm469
880494			91	111	173	167
924696	115	116	91	103	177	
939526	113	112 134	119	113	173	169
941044	121	116		113	171	161
941048	119	116	109	113	171	163
941550	117	134	119	117	173	171
941894	115	116	119	115	149 169	157
941904	115	112 116	107 119	115	169	157
942496	117	116		113	175	161
943560	117	116	109	115	173	167
943575	117	116	107	117	169	167
943576	117	116		117	169	167
951431	115	134	119	115	169	167
971897	121	120	117	101	177	
980171	115	116	107			167
9800919	119	114	101	117	173	187
9800951	99		105	111	163	169
9801709	115	134	119	115	169	167
9801888	121	110 122	101	101	183	
9801967	117	134	109	111	169	173
9900551	115	116	109	117	167	169
9900597	113	112	111	111	175	187
85-19	121	116	117	111		161
85-8	121	116	91	111	171	161
Albit	115	134	109	111	149	161
Apache	113	134	103	115	169	167

Appendix 3 (Continued).

Genotypes	gwm334	gwm389	gwm437	gwm458	gwm46	gwm469
Arlin	111	112	91	111	173	163
Armada		128 138	91	117	173	167
Arminda		134	117	115	169	169
Aztec	119	134	109	117	169	171
Baart		136	117	115	153	167
Barbee	119	106 116	101		175	165
Basin	117	116	101	115	149	167
Bezostaja	115	138	91 117	111	173 177	167
Bolero	109	138	109	117	167	163
Brevor	117 125		121	111	173	165
Bruehl		116		111	169	167
Brundage		134	109	117	173	175
Buchanan	117	116	103	111 119	175	163
Burt	119	114	109	111	173	163
C178383	128	109	113	173	167	
Cache		101	119	173	165	
Capelle	119	116	91 109	111	169	171
Capitole	115	134	91	111	177	163
Carsten	121		91	111	185	173
Cashup	115		79	111	171	163
Centurk	113	128	91	111		163
Century	113	114 126	109	111	173	163
Champln	115	138	91	117	177	167
Chey1	113	112	101	111	173	163
Chey2		112	107	111	173	163
ChinSpr	121	126	107	111	185	177

Appendix 3 (Continued).

Genotypes	gwm334	gwm389	gwm437	gwm458	gwm46	gwm469
Claire		134	109	117	173	169
Cleo	115		91 109	111	183	169
Coda	117	116	109	113	167 177	165
Consort	115	134	109		177	167
Coulee	117		91	111	171	165
Daws	117	116	101	111	171	169
Dusty	117	116	109	113	171	167
Edwin	117	110	109 119	109	175	165
Elgin	119		109	113	177	165
Elmar	119		109	111	177	163
Eltan	111	116	107	111	173	167
Etoile			117	111	171	171
Express		112	123	111	149	171
Faro	117	128	101	115	175	165
Fed1	117	116	117	111	171	
Fed2	117	116	117	111	171	187
Fidel	117	134	91	117	171	163
Foote	119	116	101	117	165	165
FR-50	115	114	91	117	173	163
Gaines	117		109	109	175	165
Gene	115			115	169	163
GldnSpike			101	111	173	159
GoldCn	117	134	119	111	173	165
Hatton	111	114 128		111	171	163
Heines	115	134	109	111	183	
Herewrd	119		91	115	177	163

Appendix 3 (Continued).

Genotypes	gwm334	gwm389	gwm437	gwm458	gwm46	gwm469
Hill81	115	116	109	111	165	167
Hiller	117		109	113	177	167
Hubbard	115 119	116	103	111	165	169
Hyb128	109		121	111 115	149	165
Hyslop	117	116	91 119	117	173	
ID377S		112	101	115	177	163
ID533	117	134	107	117	169	169
Ike	111	114	117	121	173	165
Isengrn	117	112 128	103	117	173	167
Jagger	111	134	91	115	169	167
JnsFif	111	132	111	111	157	163
Karl92	97	134	119	111	169	187
Klasic	109	128	91	115	183	171
Kmor	115	106 116	101		175	171
Lambert	119	124 134	119	111	173	171
Lewjain	117	116	109	113	171	167
LilClb	109	130	121	111 123	149	163
Luke	115	116	101		177	167
M6	119	124	85	119	177 181	165
Madsen	113	116	109	111	169	169
Malcolm	117	134	119	117	173	171
Marne	115	134	91	117	173	169
McCall	113	114	109	111 119	177	163
McDerm	117	116		111 119		167
Moisson	115	114	91	117	173	169
Moro		128	107	111	171	167

Appendix 3 (Continued).

Genotypes	gwm334	gwm389	gwm437	gwm458	gwm46	gwm469
N95L189	113	114	101	111 119	181	163
N96L1229	113	114	91	111	169	163
N97S278	111	112	117	111	177	163
N97S304	109	112	117	103 111	177	163
N97S343	113	112	117	111	177	163
Ndeprez	113	134	91	117	169	169
Norin10	121	112	103	111	173	165
Nuganz	117	124 136	109	113	175	165
NuPlnz	117	128	109	111	173	187
Nuwest			105	111	167	163
Oberkulm	121	124	95	115	173	169
Omar	115	116	119	111		165
Opata	113	112	109		187 191	
Orfed	113	114	101	111	173	187
P178383	115	128	119	115	173	167
PacBlu	119	136		115		161
Paha	117	116	109	111	173	165
Peck	115		91	111	171	165
PlnsmnV	119	136	101	111	169	167 187
Prohibit	119	134	107	117	161	165
Raeder			109	117	175	
Recital	115	112	107	113	177	169
Reman	115	112	91	117	173	167
Rex1	113	114	91	117	179	159
Rex2	111	116	105	117	179	165
Rialto		128 138	91	117	173	163

Appendix 3 (Continued).

Genotypes	gwm334	gwm389	gwm437	gwm458	gwm46	gwm469
Rndezvz	121	116	107	113	169	169
Roazon	115	128	91	117	173	169
Rod	113		109	111	175	169
Savannah	97	134	91	117	175	167
Scout66	111	112	109	111	167	163
Sideral	121	134	91	117	169	169
Soisson	115	112	119	115	175	167
Sprague	111	132	109	111 117	175	169
Sprite	109	112	101	113		173
Stephens	115		119	115	173	167
TA2460		134	119	117	147	147
TA2473			101	117	169	181
TA2524			107	117	147 173	167
Talent	113	138	91	117	167	163
Temple	117	138	101	111	173	165
Tibet	115	112	117		173 177	171
Trego	111	126	105	111 117	175	187
Tres	117		91	111		165
Triplet	119	128	95	111	169	167
Valois	121	112	91	117	169	163
Vanna	111	112		115	175	167
VM421	115	114	91	117	173	169
VM951	115	114	91 109	117	173	169
VPM1	113			117	173	169
W94481W	119	128	91	113	173	163
WA7217	119	112	91	117	175	169

Appendix 3 (Continued).

Genotypes	gwm334	gwm389	gwm437	gwm458	gwm46	gwm469
WA7437		112	109	111		165
WA7621	113	128	109		175	
WA7624			123	115	169	169
WA7666	117	116	109	117	171	167
WA7671	115		103 119	111	169	167
WA7690	117	138	101 109	119	169	167
Wanser		116	119	111	173	163
Wawawai	117	128	119	113	171	163
Wbrd906		112	91	113	173	171
Wbrd926	117		105	115	173	171
Wbrd936	115	116	107	113	179	171
Wesley		136	101	111	177	163
Weston	115	112	109	115	171	163
Winsome	115	112	109	113		169
Wthrfrd	115	116 134	109 119	117	173	169

Appendix 3 (Continued).

Genotypes	gwm513	gwm577	gwm60	gwm608	gwm95	gwm337
880494	151	128	211		120	196
924696	149		219	141	110	208
939526		150 160	211		110	192
941044		160	187	137	122	194
941048	151	160	207	139	122	188
941550	153	150	211	135	122	192
941894	153	164	187	139	120	192
941904	153	164	211	137	120	168
942496		150	187	139	122	210
943560	153	154	187	139	120	210
943575	149	160	187	139	120	192
943576	149	160	187	139	120	192
951431	147	160	207	139	122	192
971897	155		205	141	124	192
980171	153	160		139		
9800919	151	150	187	137	122	194
9800951	151	160	211		110	192
9801709	147		207	139	122	192
9801888	149		205	141	122	206
9801967	151	150	203	139	120	194
9900551	147	160	211	139	122	192
9900597	153	164	211	137	126	186 190
85-19	155	160	211	139	120	194
85-8	151	150	211	137	120	208
Albit	147	220	209	139	120	196
Apache	155		211	137	120	190

Appendix 3 (Continued).

Genotypes	gwm513	gwm577	gwm60	gwm608	gwm95	gwm337
Arlin	151	140	187	135	110	208
Armada	149	160	211	139	124	194
Arminda	155	134	223		120	192
Aztec	149			139	122	192
Baart	155	168	187	135	122	208
Barbee	145	150	211	139	122	190
Basin		130	201	139	124	180
Bezostaja	147	128	211	139	126	194
Bolero	149				122	192
Brevor	149	150	211	135	120	196
Bruehl	147		201	137	122	
Brundage	153	150		139	122	192
Buchanan	151	128	203	135	124	208
Burt	151	164	203	135	120	192
C178383	149		201	139		190
Cache	147	128			124	190
Capelle	149	160	187	137	122	194
Capitole	149	162	211	139	124	192
Carsten	149	162	203	139	122	188
Cashup	153		211		120	198
Centurk	151		211	137	122	
Century	155		211	131	126	
Champln	149	162	211		124	192
Chey1	151				122	192
Chey2	147			137	122	192
ChinSpr	145 149	130	207	137	124	190

Appendix 3 (Continued).

Genotypes	gwm513	gwm577	gwm60	gwm608	gwm95	gwm337
Claire	149	150	211		124	194
Cleo	149	160		137	122	192
Coda			211	139	120	192
Consort	149		211	139	122	192
Coulee	151	150	211	135	122	192
Daws	147	150	211	139	120	180
Dusty	149	130	201	139	122	192
Edwin	147	130	211	137	122	
Elgin	147		211	137	120	190
Elmar	147		211	137	120	190
Eltan	145	130	201	137	120	192
Etoile	149	162			120	194
Express	153	154			120	196
Faro	147	130	211	139	120	188 174
Fed1	153	128	211	137	122	210
Fed2	155	128	211	137	122	192
Fidel	149	164	187		122	192
Foote	145	150	205	139	122	192
FR-50	147	162	211	139	122	192
Gaines	151	150		135	120	198 210
Gene			211	137	110	196
GldnSpike	151	130	211		120	192
GoldCn	145		203	137	120	184
Hatton	155			135	122	
Heines	149	222	211	139	112	192
Herewrd	149	164	211	137	122	194

Appendix 3 (Continued).

Genotypes	gwm513	gwm577	gwm60	gwm608	gwm95	gwm337
Hill81	151	160	211	139	120	190
Hiller	149	150	211	137	118	190
Hubbard	149	160	203	139	120	192
Hyb128	147	222	211	139	122	190
Hyslop	153	224	211	139	124	210
ID377S	149	138	203	141	124	174
ID533	155	150		139	120	192
Ike	153	164	207		128	194
Isengrn	153		211	139	122	192
Jagger	153	162	211		120	192
JnsFif	155		211	137	120	194
Karl92	155		207		124	192
Klasic	151	154		137	110	194
Kmor	153	130		139	124	
Lambert	153	150	187	139	112	192
Lewjain	155	132	201	139	122	194
LilClb	147	130 158	211	131	128	
Luke	153	130	201	139	124	210
M6	147	154		131	110	188 194
Madsen	153	160	187	139	124	192
Malcolm	153	150	211	135	124	192
Marne	149	216	205	139	120	192
McCall	147	150	211	135	124	208
McDerm		150	211	139	124	192
Moisson	149	164		137	124	192
Moro	155	132	187	137	124	190

Appendix 3 (Continued).

Genotypes	gwm513	gwm577	gwm60	gwm608	gwm95	gwm337
N95L189	153	164	207	137	124	194
N96L1229	151	164	207	137	122	192
N97S278	153	154	187	135	110	194
N97S304	151		187	135	110	194
N97S343	153	154	187	135	124	210
Ndeprez	151				120	192 208
Norin10		132	211	139	122	196
Nuganz		150	211	135	120	210
NuPlnz	149	138	211		122	192
Nuwest	155	150			126	194
Oberkulm	147	128	205	139	128	
Omar	155	150	211		120	194
Opata	149 153	164	187	137	124	198
Orfed	155	150	211	137	122	194
P178383	155		201	139	124	194
PacBlu	149	144	187	137	126	178 192
Paha	155	150	211	137	120	
Peck	151	162	211	139	120	192
PlnsmnV	155		211	139	126	198
Prohibit	147	222	187	137	124	192
Raeder	151	150			122	198
Recital	149	162	187	139	122	210
Reman	149		211	139	122	196
Rex1	149	162	217	137	124	192
Rex2	147	128	209	139	122	208
Rialto	149	164	211	139	122	194

Appendix 3 (Continued).

Genotypes	gwm513	gwm577	gwm60	gwm608	gwm95	gwm337
Rndezvz	147	150	187	139	118	210
Roazon	149	128 216	211	139	120	192
Rod	145	140		135	124	210
Savannah	149	128	187	139	122	192
Scout66	155		211		122	210
Sideral	151		211	139	120	210
Soisson	151	130	203	137	122	190
Sprague	145	140	205	135	124	210
Sprite	147	164	211	137	122	208
Stephens	153	150	187	139	120	192
TA2460	143	130	181		122	
TA2473	143		181	137	122	188
TA2524		164	181	137	120	184
Talent	151	164	211	139	122	
Temple	155		211	137	122	198
Tibet	153	154	211	139	124	
Trego	153	164	211	137	124	192
Tres	151	150		137	120	192
Triplet	149	140	187	135	122	206
Valois	151	162	211	139	122	210
Vanna		164	211	137	110	192
VM421	149	130 216	205	137	124	192
VM951	149	216	211	137	124	192
VPM1	151	164	205	139	120	182 192
W94481W	151	216	211	137	122	208
WA7217	149	164	211	139	122	190

Appendix 3 (Continued).

Genotypes	gwm513	gwm577	gwm60	gwm608	gwm95	gwm337
WA7437	149	164	211		120	190
WA7621	145	150	205			190
WA7624	151	162	187	139	122	192
WA7666	149	164	211	139	122	192
WA7671	153	160	174		120	192
WA7690	153	130	187		120	192
Wanser	155		211		122	188
Wawawai	155	154	205	137	122	198
Wbrd906	155	154	187		110	196
Wbrd926	155	154	187	137	110	
Wbrd936	149	154	187	139	110	194
Wesley	155		207	135	122	192
Weston	151	132		137	124	196
Winsome	149	164	187		120	210
Wthrfrd	153	128 160	187	139	122	192

Appendix 4. Pedigrees of Major Cultivars with Nuclear Groupings.[b]

Cultivar	Group[a]	Release Date	Pedigree
Elgin	1a	1942	selection from Alicel, Hybrid 128/Fortyfold
Elmar	1a	1949	Hymar /3* Elgin
Omar	1a	1955	Oro // Turkey / Florence /3/3* Elgin sel. 19 /4/ Elmar
Tres	1a	1984	Suwon 92 /6* Omar /3/T. spelta / Coastal //3* Omar
Hiller	1a	1998	CI 13438 / Odin // CI 13645 / 101 /3/ M722712 /4/ Tres
Temple	1a	1997	Tres / VPM 1
Paha	1a	1970	Suwon 92 /4* Omar
Edwin	1a	2000	Jacmar/Stephens//Tres/4/PI 167822/CI 13438// Luke/3/Paha
Gaines	1b	1961	(Norin 10/Brevor, CI 13253, Sel. 14)/6/(Sel.3, CI 2692, Orfed/5/(Hybrid 50, Turkey Red/Florence//Fortyfold/ Federation/4/Oro/Turkey Red/Florence/3/Oro//Fortyfold/ Federation))/7/Burt
Nugaines	1b	1965	(Norin 10/Brevor, CI 13253, Sel. 14)/6/(Sel.3, CI 12692, Orfed/5/(Hybrid 50, Turkey Red/Florence//Fortyfold/ Federation/4/Oro//Turkey Red/Florence/3/Oro// Fortyfold/ Federation))/7/Burt
Coulee	1b	1971	Suwon 92 /4* Burt
Raeder	1b	1976	Gaines // PI 178383 / CI 13431
Stephens	1c	1977	Nord Desprez / Pullman Sel. 101 , CI 13438 CI13438 is same parent as in Luke
Hill 81	1c	1983	Yamhill / Hyslop
Hyslop	1c	1971	Nord Desprez /2*(Pullman Sel. 101 , CI 13438)
Madsen	1c	1988	VPM1/2*Hill 81
Malcolm	1c	1985	Stephens//63-189/Bezostaja
Lambert	1c	1994	Stephens / Sprague

Appendix 4 (Continued).

Cultivar	Group[a]	Release Date	Pedigree
Daws	1c	1976	CI 14484 //(CI 13645 / PI 178383 , VH66336 Sel.)
CI13645	1c	1961	Elgin 19 / Elmar // Illinois No. 1/3/ 1813
McDerm	1c	1976	Nord Desprez/7/(Pullman 101, CI13438, (Norin10/ Brevor CI 13253, Sel14)6/(Turkey Red/Florence// Fortyfold/Federation/4/Oro// Turkey Red/Florence/3/ Oro/Fortyfold//Federation, CI 12250, Sel27-15 /5/ Rio/Rex, CI 12597, sel53 .
Wthrfrd	1c	1998	Malcolm/3/VPM 1/Moisson 951//Hill/4/VPM 1/ Moisson 951 //2* Hill 81
Brundage	1c	1998	Stephens / Geneva
Luke	1d	1970	PI 178383 /2* Burt // CItr 13438
Lewjain	1d	1982	Luke/ (Luke/3/Super Helvia//Suwon 92/CI13645)
Dusty	1d	1985	Brevor / CI15923 // Nugaines
Kmor	1d	1990	Luke/VH067375/4/*Ae. ventricosa*/Triticum persicum/ Marne *3/3/Moisson
Coda	1d	1998	Tres / Madsen // Tres
Barbee	2a	1976	Omar / Vogels 1834-Sel . 3// PI 178383 / CI 13431
Capelle	2a	1946	Vilmorin 27/Hybride du Joncquois
NDeprez	2a		Vilmorin 27/Hybride de Jonquois
Etoile	2a	1950	Mon Desir/Ardito//Mouton a Epi Rouge/K3/3/ Mouton a Epi Rouge
Isengrn	2a	1978	TP 229//Perdix/Hybrid 46/3/TP 118//Perdix/ Hybrid 46/4/Cappelle Desprez/Champlein/3/Viking/ Tetrix //Tetrix/Jubilegem
Talent	2a	1973	Champlein /3/ Thatcher / Vilmorin 27 // Fortunato

Appendix 4 (Continued).

Cultivar	Group[a]	Release Date	Pedigree
Renan	2a	1992	Mironovskaja 80/Maris Huntsman//VPM/Moisson// (Norin 10/Brevor/B21)
VPM1	2b		*Ae. ventricosa/T.persicum* //3* Marne
Roazon	2b	1976	VPM 1/Moisson
Moisson	2b	1963	Capelle Desprez // Hybride 80-3 / Etoile de Choisy
Capitole	2b	1964	Capelle Desprez // 80 / Etoile de Choisy
Champlein	2b	1956	Tapedi/(Vilmorin 27 / Red Fife)
Cheyenne	2c	1933	Selection from Crimean, CI 1435
Scout	2c	1964	Nebred // Hope / Turkey /3/ Cheyenne / Ponca
Wanser	2c	1965	Burt / (Blackhull / Rex // Cheyenne)
Century	2c	1986	Payne//(Norin10/3/Nebraska 60//Mediterranean/Hope /4/ Bison)/ Amigo
Hatton	2c	1979	PI 142522 /2* McCall
Brevor	3	1949	Turkey Red/Florence//Fortyfold/Federation,CI 11912/4/ Oro/Turkey Red/Florence/3/Oro//Fortyfold / Federation
McCall	3	1965	Burt/ (Blackhull / Rex // Cheyenne)
Arlin	4a	1992	Pedigree unknown
N97S278	4a		Arlin/Pronghorn
N97S304	4a		Arlin/Pronghorn
N97S343	4a		N91L122/Arlin
Burt	4c	1956	Cross 27-15//Rio/Rex
PlainsmanV	4c	1974	SRAI 1970 / SRAI 1900
Karl 92	4c	1992	Plainsman V /3/ Kaw / Atlas 50 // Parker *5/ Agent
Wesley	4c	1998	KS831936-3/NE86501 = Sumner sib(Plainsman V/

Odesskaya 51)//Colt/Cody

Appendix 4 (Continued).

[a] 1.Pacific Northwest Soft White Cultivars.

1a. Club Wheats and SW Foundation Cultivars.

1b. Gaines Lineage Based on Norin 10, Brevor, and Burt.

1c. Stephens Lineage Based on Nord Desprez and Pullman 101.

1d. Luke Lineage Based on Modern Derivatives of Norin10/ Brevor.

2. European and Related Red Wheat Cultivars.

2a. European-derived Cultivars with Vilmorin 27-based Lineage.

2b. Parents and Derivatives of VPM1.

2c. Foundation Cultivars for Great Plains Hard Red Winter.

3. Distantly Related Cultivars.

4. Great Plains Hard Wheat Cultivars.

4a. Hard White Winter Lines Based on Arlin.

4c. Modern Hard Red Winter Lineage with Contributions from PlainsmanV.

[b] Pedigrees were obtained from the National Plant Germplasm System.

Appendix 5. Primer locations and sequences.

Marker	Location [a]	Sequence
Xgwm3	3D	5' AATATCGCATCACTATCCCA 3'
		5' GCAGCGGCACTGGTACATTT 3'
Xgwm18	4B	5' GGTTGCTGAAGAACCTTATTTAGG 3'
		5' TGGCGCCATGATTGCATTATCTTC 3'
Xgwm46	7B	5' GCACGTGAATGGATTGGAC 3'
		5' TGACCCAATAGTGGTGGTCA 3'
Xgwm60	7A	5' GCATTGACAGATGCACACG 3'
		5' TGTCCTACACGGACCACGT 3'
Xgwm95	2A	5' AATGCAAAGTGAAAAACCCG 3'
		5' GATCAAACACACACCCCTCC 3'
Xgwm135	1A	5' ACACTGTCAACCTGGCAATG 3'
		5' TGTCAACATCGTTTTGAAAAGG 3'
Xgwm148	2B	5' CAAAGCTTGACTCAGACCAAA 3'
		5' GTGAGGCAGCAAGAGAGAAA 3'
Xgwm155	3A	5' AATCATTGGAAATCCATATGCC 3'
		5' CAATCATTTCCCCCTCCC 3'
Xgwm160	4A	5' CTGCAGGAAAAAAAGTACACCC 3'
		5' TTCAATTCAGTCTTGGCTTGG 3'
Xgwm169	6A	5' ACCACTGCAGAGAACACATAC 3'
		5' ACCACTGCAGAGAACACATACG 3'
Xgwm190	5D	5' GTGCCACGTGGTACCTTTG 3'
		5' GTGCTTGCTGAGCTATGAGTC 3'
Xgwm194	4D	5' CGACGCAGAACTTAAACAAG 3'
		5' GATCTGCTCTACTCTCCTCC 3'
Xgwm234	5B	5' CTCATTGGGGTGTGTACGTG 3'
		5' GAGTCCTGATGTGAAGCTGTTG 3'

Appendix 5 (Continued).

Marker	Location [a]	Sequence
Xgwm261	2D	5' CTCCCTGTACGCCTAAGGC 3'
		5' CTCGCGCTACTAGCCATTG 3'
Xgwm282	7A	5' TCTCATTCACACACAACACTAGC 3'
		5' TTGGCCGTGTAAGGCAG 3'
Xgwm334	6A	5' AACATGTGTTTTTAGCTATC 3'
		5' AATTTCAAAAAGGAGAGAGA 3'
Xgwm337	1D	5' CCTCTTCCTCCCTCACTTAGC 3'
		5' TGCTAACTGGCCTTTGCC 3'
Xgwm389	3B	5' ATCATGTCGATCTCCTTGACG 3'
		5' TGCCATGCACATTAGCAGAT 3'
Xgwm437	7D	5' GATCAAGACTTTTGTATCTCTC 3'
		5' GATGTCCAACAGTTAGCTTA 3'
Xgwm458	1D	5' AATGGCAATTGGAAGACATAGC 3'
		5' TTCGCAATGTTGATTTGGC 3'
Xgwm469	6D	5' CAACTCAGTGCTCACACAACG 3'
		5' CGATAACCACTCATCCACACC 3'
Xgwm513	4B	5' ATCCGTAGCACCTACTGGTCA 3'
		5' GGTCTGTTCATGCCACATTG 3'
Xgwm577	7B	5' ATGGCATAATTTGGTGAAATTG 3'
		5' TGTTTCAAGCCCAACTTCTATT 3'
Xgwm608	4D	5' ACATTGTGTGTGCGGCC 3'
		5' GATCCCTCTCCGCTAGAAGC 3'
WCt1	Ct	5' CATCCTTTTCAATCCAAAATCA 3'
		5' GATTAGTGCCGGATACGGG 3'
WCt4	Ct	5' TCTTCGGAAACGGAAAACC 3'
		5' GGATTTCCCATTATGGGTCC 3'

Appendix 5 (Continued).

Marker	Location [a]	Sequence
WCt6	Ct	5' TCACAGGCTGCAAAATTCAG 3'
		5' GGATAATAATGCTGTTCGGACC 3'
WCt8	Ct	5' CTTGGGCAACTGCGTAAATT 3'
		5' ACCAAGAAAGCACATCAGATCA 3'
WCt9	Ct	5' CGCAGCCTATATAGGTGAATCC 3'
		5' TTGCAACCAAGCAGATTATCC 3'
WCt10	Ct	5' TGCCCTTTTTTAACCAATGC 3'
		5' CATGGTCAGCAAAGTTGTTTC 3'
WCt11	Ct	5' TTTTATCTAGGCGGAAGAGTCC 3'
		5' TCATTTGGCTCTCACGCTC 3'
WCt12	Ct	5' CGATCCCTATGTAGAAAGCCC 3'
		5' AACGAAACCCCTTCTTACCG 3'
WCt13	Ct	5' TGAAAATCCTCGTGTCACCA 3'
		5' TGTATCACAATCCATCTCGAGG 3'
WCt14	Ct	5' TCAACAAGTGACTCGAACTGTG 3'
		5' CGTCATGGAATAGGTGTCTCA 3'
WCt15	Ct	5' CAATCTGGTTTTGCCTGGTT 3'
		5' ATGGGGTTTTCTATTGATGCC 3'
WCt17	Ct	5' GATCGTTCACTCCAAAAAGAGG 3'
		5' ACCCCATTGAATGAAAAAATG 3'
WCt18	Ct	5' TGATTCGGAATTAGGGACTCA 3'
		5' GTAAGCATGAAAGAGTTAAATTCCA 3'
WCt19	Ct	5' TTTGGAAAAAATAAGTCTCTTCGC 3'
		5' GCGTATCGAAGACTCGAAGG 3'
WCt22	Ct	5' GCAATAGTGTCCTTGCCCAT 3'
		5' ACCAAAATAGTTTCATTAGCTCCTG 3'

[a] Ct is wheat chloroplast.