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AFLP and pedigree-based genetic diversity estimates in modern cultivars of durum wheat [*Triticum turgidum* L. subsp. *durum* (Desf.) Husn.]

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Abstract A substantial amount of between and within cultivar genetic variation was detected in all the 13 registered modern Canadian durum wheat (*Triticum turgidum* L. ssp. *durum* (Desf.) Husn.) cultivars based upon amplified restriction fragment polymorphism (AFLP). Of the approximately 950 detected AFLP markers, only 89 were polymorphic, with 41 between cultivars whereas the remaining 48 showed polymorphism within at least one cultivar. The ancestry of Canadian durum wheat cultivars was traced back to 125 cultivars, selections, and breeding lines including 17 landraces. Mean pairwise genetic distance based on the kinship coefficient was 0.76. On the other hand, AFLP-based mean pairwise genetic distance was 0.40. Even though there was a large difference between the means of the two diversity measures, a moderate positive correlation (r=0.457, p<0.002) was detected between the two distance matrices. Cluster analysis with the entire AFLP data divided all cultivars into three major groups reflecting their breeding origins. One group contained 'Pelissier' alone, which was a selection from a landrace introduced into the US from Algeria. On the other hand such groupings among cultivars were not evident when KIN was used for genetic diversity measures instead. The level of genetic variation among individuals within a cultivar at the breeders' seed level was estimated based on an inter-haplotypic distance matrix derived from the AFLP data. We found that the level of genetic variation within the most-developed cultivars is fairly substantial despite rigorous selection pressure aimed at cultivar purity in breeding programs. Comparison of AFLP and pedigree-based genetic diversity estimates in crop species such as durum wheat can provide important information for plant improvement.

Keywords Amplified restriction fragment polymorphism (AFLP) · Kinship coefficient (KIN) · Genetic diversity estimates · Durum wheat

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Introduction

Measurements of genetic diversity in cultivated crops have important implications for breeding programs and for the conservation of genetic resources. Both pedigree (Cox et al. 1985; Martin et al. 1991) and DNA markers (Karp et al. 1997; Barrett et al. 1998; Davila et al. 1998) have been used to measure genetic diversity levels among genotypes. In self-pollinating crop species with a known pedigree, the kinship coefficient (KIN) (Tinker et al. 1993) can be used as an inexpensive tool for genetic diversity estimates. KIN is the probability of identity by descent for a particular allele between two genotypes. It gives a theoretical measure of genetic diversity/relatedness among cultivars based on the assumption of equal parental contribution. Pedigree-based diversity measurements can result in an overestimation of the actual level of genetic diversity present in the gene pool as a result of the assumptions that are made regarding genetic drift, selection pressure, and the relatedness of ancestors without a known pedigree (Cox et al. 1985; Graner et al 1994; Kim and Ward, 1997; Barrett et al. 1998).

DNA markers have the advantage of directly detecting sequence variation among cultivars and therefore the ability to bypass the assumptions that are inherent to pedigree analysis. Autrique et al. (1996) identified Restriction fragment length polymorphisms (RFLPs) in 113 improved cultivars and landraces of durum wheat and reported a mean genetic distance of 0.21 and 0.31 within the improved lines and landraces respectively. In bread wheat (*T. aestivum* L.), a mean genetic distance of 0.54

was reported by Barrett et al. (1998) with AFLP markers.

Correlation between DNA markers and pedigree-based diversity estimates has varied from 0.21 between RFLP and Pedigree methods in barley (*Hordeum vulgare* L.) (Graner et al. 1994) and durum wheat (Autrique et al. 1996) to 0.81 between RFLP and pedigree data among 37 maize (*Zea mays* L.) inbred lines (Smith et al. 1990). A moderate correlation coefficient value of 0.42 was found between AFLP and pedigree data in bread wheat cultivars from the Pacific Northwest (Barrett et al. 1998).

DNA markers are most suitable for genetic diversity estimates (O'Donoughue et al. 1994; Kim and Ward 1997; Plaschke et al. 1995; Ahnert et al. 1996; Davila et al. 1998), however, the extent of their utility may depend on the nature of the marker, their number, genome coverage, and the population under investigation. DNA-based markers are particularly useful for wheat or other crop species with a narrow genetic basis. Markers that can detect higher levels of polymorphism between wheat varieties can be utilized to derive more-accurate genetic diversity estimates. The objective of this study was to investigate the utility of AFLP fingerprinting for estimating the levels of genetic diversity in a modern crop with an apparent narrow genetic variation, i.e., Canadian durum wheat cultivars, and to compare AFLP and pedigree-based genetic diversity estimates. As with RFLP analysis, AFLP results from mutations that alter restriction endonuclease recognition sites. AFLP technology is highly reproducible and, in addition, its multiplex ratio can be changed simply by altering the number of selective nucleotides at the 3′ end of PCR primers. We have exploited these two properties in this study of genetic diversity in a species with a narrow genetic base, namely durum wheat.

Materials and methods

Plant materials

Breeder's seeds from 13 cultivars (Table 1) were obtained from the Canadian Food Inspection Agency, Ottawa. Ten seeds from each cultivar were grown in a phytotron at 22°C for 3 weeks and 1–2 g of fresh leaf material was harvested for DNA extraction.

DNA extraction and AFLP analysis

DNA from 130 plants representing 13 cultivars (ten plants per cultivar) was isolated by a modified CTAB procedure (Doyle and Doyle 1987). Working solutions of 50 ng/µl were prepared by dilution of the original stock solution in TE (10 mM Tris-HCl, pH 7.5, 0.1 mM EDTA) buffer. Five hundred nanograms of DNA from each plant were digested with *Tru*9I (an isoschizomer of *Mse*I) and *Eco*RI restriction enzymes. Adapters of known sequence were ligated to the ends of the restricted fragments with T4 DNA ligase (Promega). The AFLP method has been extensively described by Vos et al. (1995). Amplification of the restriction fragments was carried out using two sets of primers. The *Eco*RI primer sets (E) all contain the core sequence 5′GACTGCGTACCAATTC, while all the *Mse*I primers (M) have the sequence 5′GATGAGTCCTGAGTAA in common. AFLP-PCR amplifications were carried out in two steps: a pre-amplification PCR reaction with E+A and M+C primers (where the symbol following the E or M represents an additional selective nucleotide at the 3′ end of each primer and N represents one of the four possible nucleotides) was performed to reduce the number of amplified restriction fragments by a factor of 16. Subsequently, the product of the pre-amplification reaction was used as template for selective amplification using ^{33}P-labelled E+ANN in combination with M+CNN primers resulting in a calculated 4,096-fold overall reduction in the number of generated PCR fragments. The PCR products were mixed with an equal volume of denaturing dye (98% de-ionized formamide, 0.025% bromo-phenol-blue, 0.025% xylene cyanol) and denatured at 94°C for 3 min. Amplification products were resolved in a 5% denaturing polyacrylamide gel prepared with 1×TBE (100 mM Tris, 100 mM boric acid, 2 mM EDTA, pH 8.0) as the running buffer and electrophoresed at 80 W (constant power) for 2.5 h. The gels were dried and exposed to Kodak X-Omat film for 2–3 days. DNA fingerprints were evaluated by visual inspection of autoradiographs.

Table 1 The 13 cultivars used in this genetic diversity study of durum wheat

Cultivar Name	Code	Origin
'AC Avonlea'	AVN1	SCRS[a]
'AC Melita'	MEL1	WRS[b]
'AC Morse'	MRS1	WRS
'AC Navigator'	NAV1	SCRS
'AC Pathfinder'	PAT1	SCRS
'Hercules'	HRC1	WRS
'Kyle'	KYL1	SCRS
'Medora'	MED1	WRS
'Pelissier'	PLS1	Selection from a landrace (Algeria)
'Plenty'	PLN1	University of Saskatchewan
'Sceptre'	SCP1	University of Saskatchewan
'Wakooma'	WAK1	SCRS
'Wascana'	WAS1	SCRS

[a] Semiarid Prairie Agricultural Research Centre, Swift Current, Saskatchewan, Canada
[b] Cereal Research Centre, Winnipeg, Manitoba, Canada

Data acquisition and diversity analysis

In this work each band was considered to be a single locus. Autoradiographs were scored based on the presence or absence of bands at polymorphic loci generating a matrix of 1 s and 0 s representing the presence and absence of a band respectively. Only those bands falling within the range of 50–500 bp were considered. These bands were used to generate a genetic similarity matrix with the SIMQUAL routine DICE based on Dice's (1945) formula, from the NTSYS-pc statistical package (Rohlf 1990). The genetic similarity matrix was transformed into a distance matrix using the TRANSF subroutine of NTSYS-pc, resulting in a distance matrix equivalent to Nei and Li's (1979) genetic distance matrix. Clustering of genotypes was performed using the SAHN in NTSYS-pc based on the genetic distance matrix with the UPGMA method. Within-cultivar genetic diversity levels were estimated based on the number of pair-wise differences between pairs of haplotypes as measured by the average gene diversity per locus using the Arlequin program (Scheider et al. 1997).

Pedigree analysis

Cultivar descriptions of Canadian durum wheat cultivars were obtained from the Canadian Food Inspection Agency. Information regarding their known ancestors was obtained from cultivar de-

scriptions, breeding records, personal communication with breeders, published pedigree databases (Zeven and Zeven-Hissink 1976) and the Germplasm Resource Information Network (available at URL *http://www.ars-grin.gov*). The ancestry of each cultivar was traced as far back as possible. For each cultivar a pedigree was constructed using the Cultivar Registry System software package (Baum et al. 1990). Kinship Coefficients (r) were calculated using the Bigkin87 program (Tinker and Mather 1993) based on the following assumptions: (1) all ancestors, cultivars, and breeding lines are homozygous and homogeneous; (2) cultivars without a known pedigree are unrelated; (3) parents make an equal contribution of alleles to each progeny; and (4) the relationship between an ancestor and a line derived from that ancestor was r=0.75. By using pedigree information we were able to account for the selfing and backcrosses in Bigkin87. Pair-wise r values were used to generate a kinship coefficient matrix. The TRANSF subroutine of NTSYS-pc was used to transform this matrix into a distance matrix based on (1-r). Clustering of cultivars was performed as in the previous section "diversity analysis".

Matrix comparison

The AFLP-based Nei and Li's genetic distance matrix and the pedigree-based distance matrix based on the kinship coefficients were compared by the MAXCOMP routine of NTSYS-pc. The normalized Mantel statistic Z (Mantel 1967) was used to determine the level of association between the two matrices. The COPH subroutine of NTSYS-pc was used to determine the cophenetic correlation coefficient between the similarity matrix and the similarity cluster for the two AFLPs and the pedigree data sets. The statistical considerations for these analyses were described by Beer et al. (1993).

Table 2 The number of scored polymorphic markers detected by AFLP analysis within and among the 13 Canadian durum wheat cultivars

Primer pair	Cultivar-specific	Polymorphic within cultivar	Total
EAAG/MCGC	7	5	12
EAAG/MCAG	8	7	15
EAAC/MCTA	6	6	12
EAGC/MCAG	6	8	14
EAGC/MCTT	3	7	10
EATG/MCTC	7	4	11
EAAG/MCCG	0	5	5
EAAC/MCTC	0	3	3
EAGG/MCTG	3	2	5
EAGG/MCTC	1	1	2
Total	41	48	89

Analysis of molecular variance (AMOVA)

The matrix of the 130 genotypes was summarized into haplotypes. The reduced matrix of haplotypes was then submitted to Arlequin (Scheider et al. 1997) to compute the genetic structure, i.e., inter-haplotypic distances within and between cultivars, average gene diversity over loci, variance components and related statistics, all obtained from the AMOVA module.

Results

AFLP analysis

Ten AFLP primer pairs were sufficient to detect 89 polymorphic markers among 130 genotypes resulting in an average of 8.9 polymorphic loci per primer pair (Table 2). Forty one markers showed cultivar-specific polymorphism, i.e., either present or absent within all ten plants in each cultivar. The pair-wise genetic distance matrix for the 41 cultivar-specific markers is presented in Table 3 (lower triangle). The remaining 48 markers showed polymorphism both within and among cultivars. An example of an AFLP autoradiograph, showing both polymorphism within and among cultivars, is shown in Fig. 1. There was no linear relationship between the number of amplified loci with the number of polymorphic markers for any given primer pair (data not shown). Average genetic distance between pairs of cultivars was estimated at 0.40 when only the cultivar-specific markers were used. This value dropped to 0.38 when all the polymorphic markers were pooled in the analysis.

Clustering of genotypes based on the cultivar-specific AFLP markers resulted in two distinct clusters at the phenon line of 0.48 (Fig. 2). The first cluster contains 'AC Avonlea', 'AC Melita', 'Medora', 'Sceptre', 'Hercules', 'Plenty' and 'AC Morse'. The second cluster contains 'AC Navigator', 'AC Pathfinder', 'Kyle', 'Wakooma', 'Wascana' and the selection from a landrace 'Pelissier'.

Table 3 Matrix of pair-wise genetic distances between Canadian durum wheat cultivars based on kinship coefficients (above diagonal) and on Nei and Li's formula (below diagonal). AFLP-based genetic distances were obtained from the 41 cultivar-specific markers

Cultivar	1	2	3	4	5	6	7	8	9	10	11	12	13
1 AC Avonlea	0.0000	0.7830	0.6970	0.8680	0.6860	0.8480	0.8490	0.7010	0.9770	0.8770	0.9130	0.8460	0.8140
2 AC Melita	0.3617	0.0000	0.5830	0.7550	0.8420	0.6660	0.7740	0.3280	1.0000	0.7390	0.8040	0.7870	0.7870
3 AC Morse	0.3636	0.3191	0.0000	0.7930	0.7510	0.6700	0.7960	0.4380	0.9880	0.7840	0.7770	0.8010	0.7860
4 AC Navigator	0.4500	0.4419	0.5500	0.0000	0.6310	0.8120	0.3820	0.7580	0.9410	0.7820	0.9020	0.4740	0.5210
5 AC Pathfinder	0.4500	0.4419	0.5000	0.2778	0.0000	0.8890	0.7950	0.8410	0.9650	0.8730	0.9390	0.7890	0.7420
6 Hercules	0.4667	0.3333	0.4222	0.5122	0.5610	0.0000	0.7990	0.5690	1.0000	0.6420	0.7680	0.8110	0.8110
7 Kyle	0.4737	0.5122	0.6842	0.1765	0.4118	0.5385	0.0000	0.7820	0.9060	0.7340	0.9030	0.1840	0.3090
8 Medora	0.2917	0.1765	0.2500	0.4545	0.5000	0.2245	0.3810	0.0000	1.0000	0.7290	0.7410	0.7950	0.7950
9 Pelissier	0.4500	0.5814	0.6000	0.2778	0.3333	0.6585	0.3529	0.6364	0.0000	0.9530	1.0000	0.8120	0.8120
10 Plenty	0.3778	0.2917	0.3778	0.4634	0.4634	0.2174	0.4872	0.2653	0.5610	0.0000	0.8840	0.7240	0.6620
11 Sceptre	0.3636	0.2766	0.3182	0.4000	0.5000	0.2444	0.3684	0.1667	0.6000	0.2000	0.0000	0.9090	0.9090
12 Wakooma	0.3333	0.4222	0.4762	0.3158	0.5263	0.4419	0.2222	0.3478	0.4211	0.3488	0.2381	0.0000	0.2500
13 Wascana	0.4419	0.4348	0.4419	0.3333	0.2308	0.5000	0.4054	0.4043	0.3333	0.4091	0.4419	0.4634	0.0000

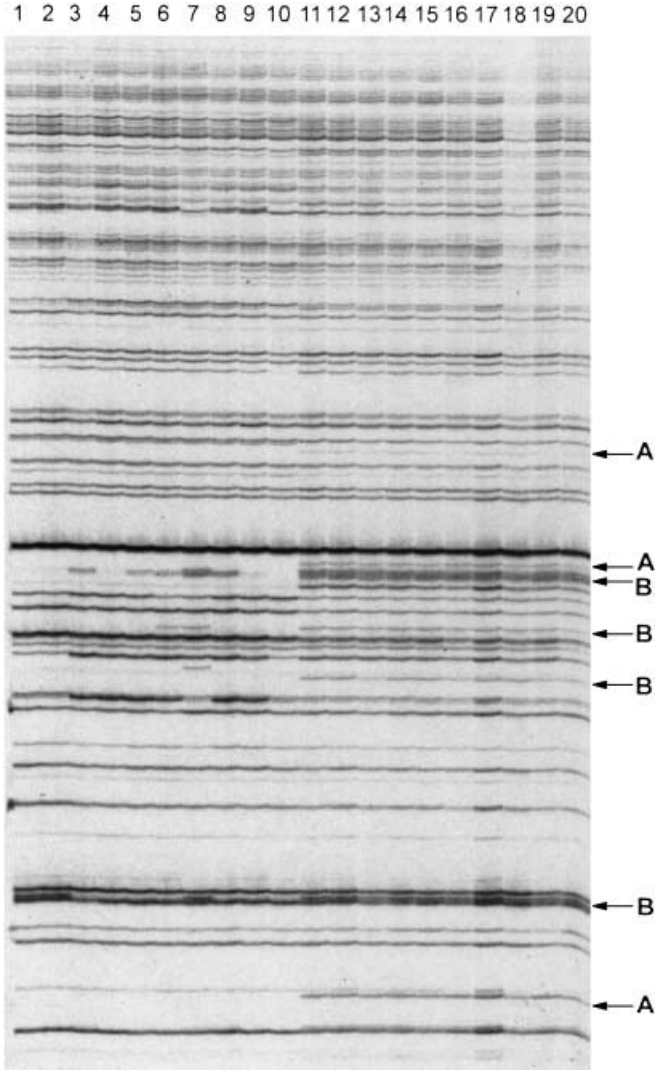

Fig. 1 Autoradiogram of a portion of an AFLP fingerprint using the EAAG/MCAG primer pair demonstrating polymorphisms. *Lanes 1–10* 'Pelissier', *11–20* 'Medora'. "*A*" indicates cultivar-specific markers and "*B*" within-cultivar polymorphic markers

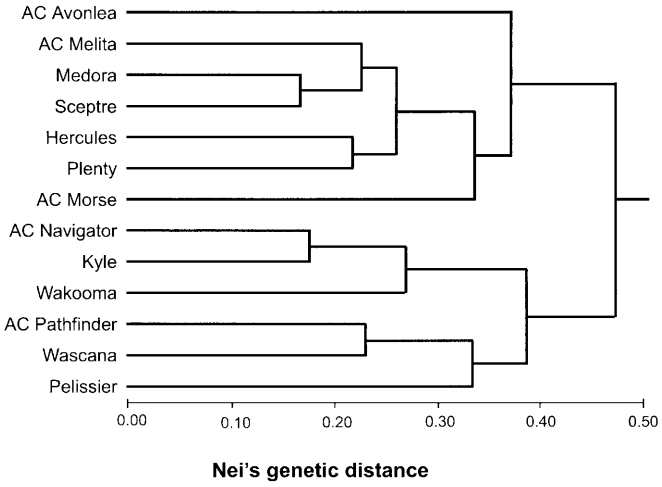

Fig. 2 UPGMA dendrogram showing genetic relationships among the 13 durum wheat cultivars used in this study. The dendrogram was constructed using 41 cultivar-specific AFLP markers and was based on the genetic distance calculated according to Nei's genetic coefficient

The AFLP data set was first split into two subsets, a subset of 41 cultivar-specific markers (Fig. 2) and a subset consisting of the 48 markers polymorphic within cultivar fingerprints (data not shown). Cluster analysis was performed on each data set alone as well as the entire AFLP data to see if there was any difference in the clustering and in the genetic relationships among cultivars in each data subset. Clustering of genotypes with the two data subsets and the entire data set separately recovered cultivars developed at the Winnipeg Research Station and the University of Saskatchewan in one group, and cultivars developed at the Swift Current research Station in the second group. When the entire data set (the two subsets together) was input into the cluster analysis all individual plants from each cultivar remained grouped together under each cultivar (Fig. 3). However, when the subset of the 48 within-cultivar polymorphic AFLP markers was used all, except for two individual plants one from 'AC Morse' and one from 'Pelissier', clustered together into their cultivar grouping.

The level of genetic diversity/homogeneity within each cultivar was estimated from the 48 AFLP markers that showed polymorphism within cultivars. The 130 single plants in our study fell into 104 unique haplotypes. Based on the inter-haplotypic distances within cultivars, 'Pelissier' and 'Wascana' showed the highest and the lowest levels of genetic heterogeneity respectively (Fig. 4). The level of within-cultivar genetic heterogeneity, obtained from the average gene diversity over loci, is shown in Fig. 4 as an increasing trend from the cultivar with the lowest.

Pedigree analysis

The ancestry of Canadian durum wheat cultivars was traced back to 125 cultivars and breeding lines including 17 landraces. We found that 11 ancestral lines and cultivars were present in at least 85% of the pedigrees and that three of them, 'Vernal Emmer', 'Mindum' and 'Heiti', were present in all pedigrees. The pair-wise genetic distances based on KIN is presented in Table 3 (upper triangle). The average pair-wise genetic distance between pairs of cultivars based on KIN was 0.76. Clustering of genotypes based on KIN did not result in any major groupings. At the 0.75 phenon line five clusters were evident. 'AC Avonlea' and 'AC Pathfinder' in the first cluster, 'AC Melita', 'Medora', 'AC Morse', 'Hercules' and 'Plenty' in the second cluster, with 'AC Navigator', 'Kyle', 'Wakooma' and 'Wascana' in the third cluster. 'Sceptre' and 'Pelissier' formed the fourth and fifth cluster respectively (Fig. 5).

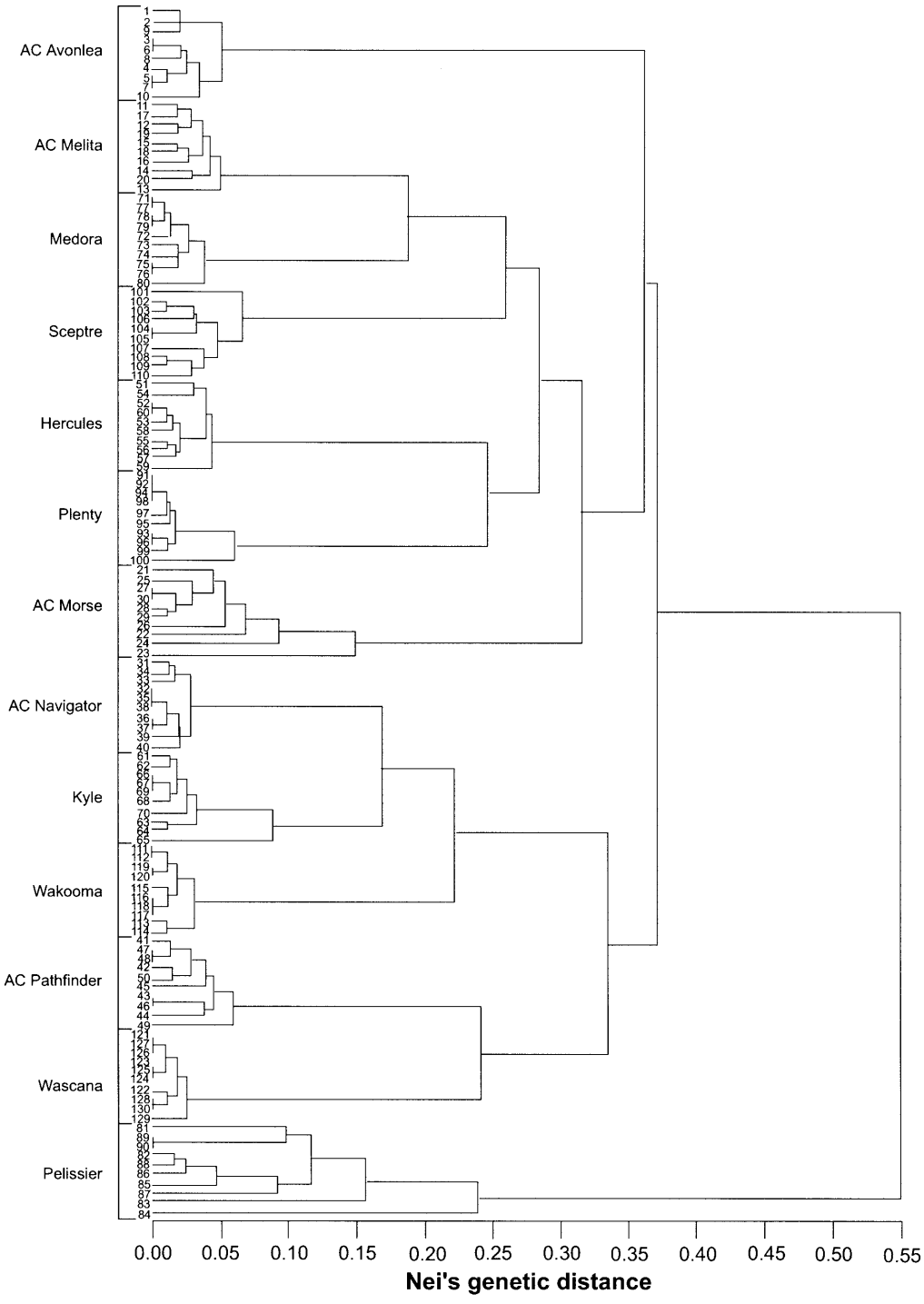

Fig. 3 UPGMA dendrogram depicting genetic relationships among 130 durum wheat plants representing 13 cultivars. The dendrogram was constructed using 89 AFLP markers and was based on the genetic distance calculated according to Nei's genetic coefficient

Matrix comparison and Mantel test

The degree of relationship between the distance matrices based on AFLP and KIN was measured by comparing the distance matrices with the normalized Mantel statistics. A moderately positive correlation (r=0.457, p<0.002) between the two matrices was observed. The goodness of fit, determined by the correlation between the cophenetic matrix and the distance matrix as described, between the pedigree graph and the distance matrix from KIN was 0.94, between the UPGMA dendrogram of the 130 individual plants using all the markers and the distance matrix of the same was 0.91, and the UPGMA dendrogram using only the cultivar-specific markers and the distance matrix was0.77.

Analysis of molecular variance (AMOVA)

The matrix of genotypes was summarized to a matrix of 104 haplotypes. The genetic structure is summarized in

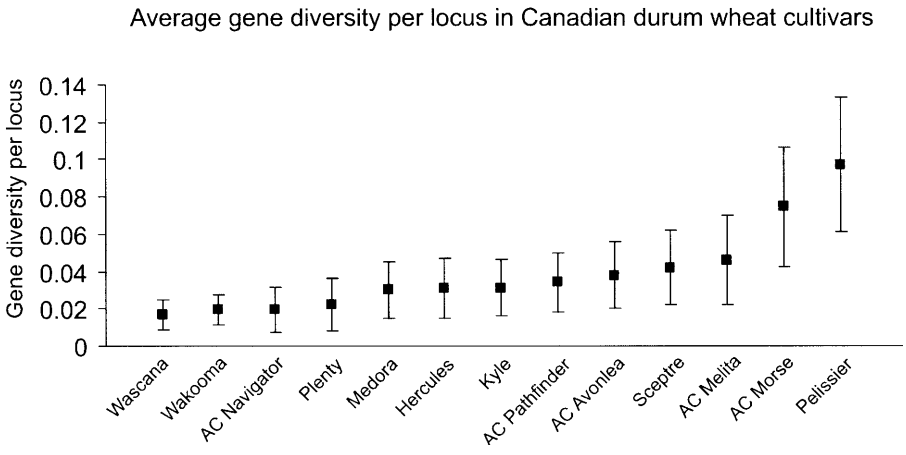

Fig. 4 Comparison of the genetic diversity per locus among the individual plants within each cultivar based on inter-haplotypic distances. Mean: *solid squares*; standard error: *lines*

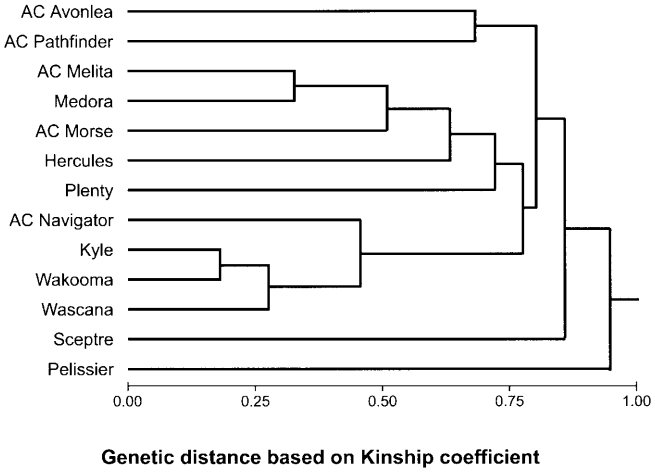

Fig. 5 UPGMA dendrogram showing genetic relationships among the 13 durum wheat cultivars used in this study. The dendrogram was constructed using the matrix of kinship coefficients

Table 4 Analysis of molecular variance (AMOVA).

Source of variation	*df*	Sum of squares	Variance components	Percentage of variation
Among populations	12	1,781.446	14.67239 Va[a]	89.45
Within populations	117	202.400	1.72991 Vb[b]	10.55
Total	129	1983.846	16.40231	100.00

[a] Va: among-populations (cultivars) variance
[b] Vb: within-populations variance

Table 4. The variance component between cultivars is about 8.5-fold larger than the within-cultivars variance component.

Discussion

Clustering of Canadian durum wheat cultivars based on 89 polymorphic AFLP markers revealed a low level of genetic variation compared to the estimates obtained by pedigree analysis. The AFLP analysis based on cultivar specific-markers and pedigree-based mean pair-wise genetic distances were 0.40 and 0.76 respectively. The moderate correlation coefficient (r=0.45) found between matrices based on AFLP genetic diversity estimates and KIN is similar to that reported for coefficient of parentage (COP) and AFLP in hexaploid wheat (Barrett et al. 1998), but higher than those obtained for durum wheat using RFLP and pedigree data (Autrique et al. 1996). On the other hand this value is lower than those obtained for maize (Smith et al. 1990). In the present study the computation of genetic distances was restricted to the polymorphic markers. Although we had detected approximately 950 loci, only 9% were polymorphic and used in the computation. Exclusion of those monomorphic loci did not change the clustering and the correlation results, because these loci are non-informative. However, this exclusion overestimates the mean pairwise genetic distances. Meaningful comparisons focused on differences alone, i.e. polymorphic bands alone, emphasize the degree of diversity between the cultivars in the study.

The discrepancy between pedigree and AFLP-based genetic diversity estimates may be the result of assumptions that were made in the calculation of genetic relatedness based on pedigrees. (Souza and Sorrells 1989; Cox and Murphy 1990; Martin et al. 1991; Tinker et al. 1993).These estimates may be biased due to selection pressure, unequal parental contribution, and the relatedness of ancestors without a known pedigree. The assumption of no genetic relationship between ancestors without a known pedigree maybe a major factor contributing to the disparity between pedigree and molecular marker-based diversity estimates especially when comprehensive pedigree information in not available. In the comparison of AFLP and pedigree-based genetic distances it was necessary to use only the cultivar-specific markers, i.e., the 41 monomorphic within cultivars. An RFLP analysis of two ancestral durum cultivars (‘Khapli’ and ‘Vernal Emmer’), which are also present in the parentage of most Canadian durum cultivars, by Autrique et al. (1996) showed that they were different for only two out of the 134 alleles that were assayed.

This results demonstrates that pedigree-based diversity measurements may result in an overestimation of the actual levels of genetic diversity.

Lower genetic variation measured at the DNA level among modern cultivars is most likely the result of selection pressure and genetic drift in breeding programs. Such practices, which are aimed at genetic homogenization and purity of cultivars, may result in an improvement in yield and other agronomically important traits at the expense of reduction in the genetic base of crops.

UPGMA cluster analysis using 41 AFLP markers (polymorphic between cultivars but monomorphic between the individual plants within cultivars) produced two clusters (Fig. 2). And the addition of 48 AFLP markers that were polymorphic between the individual plants within cultivars to the data set did not produce any major structural rearrangements in the dendrogram, except that 'Pelissier', an early selection from a landrace, fell into a distinct cluster (Fig. 3). When the subset of 48 within-cultivar polymorphic AFLP markers was used 2 out of 130 plants were placed in the wrong cultivar groups (data not shown). This result was expected given the relatively higher level of heterogeneity that was detected among individual plants within these two cultivars compared to the corresponding levels in other cultivars (Fig. 4). Another cause for this result may be impurities in the 'breeders seed' material, although at this level the seed material is expected to be pure. Another possibility could be due to a very small degree of outcrossing, as reported for bread wheat in Western Canada, 0.2–4% (Hucl 1996), and higher rates, up to 16%, in some local Russian durum wheat cultivars (Pyl'nev 1979); an appreciable amount of outcrossing was also detected among tetraploid wheat landraces collected from the central highlands of Ethiopia (Tsegaye 1997).

The relative consistency in the results of using different subsets of AFLP marker data suggests that an adequate number of markers were used over the whole range of the genome although the nature and genomic locations or distribution of our markers are unknown; in a different study Peng et al. (2000) found that the distribution of AFLP markers was not random and most were clustered near the centromeric regions. When data from individual primer combinations were analysed separately there were apparent differences among the dendrograms (not dealt with in the Results section). We successively added data from different primer combinations until no significant change in the dendrogram was obtained. Stability in the results was observed after the combination of data from five primer pairs. However, data from ten primer pairs combined was required to fully discern the inter-haplotypic differences.

Pairs of cultivars such as 'Kyle' and 'AC Navigator' that were closely related in the pedigree-based dendrogram (Fig. 5) were more-closely related in the AFLP-based dendrogram (Fig. 2). On the other hand 'Wakooma' and 'Wascana' clustered closely in the pedigree dendrogram (Fig. 5) but much less so in the AFLP-based dendrogram (Fig. 2). 'Sceptre' and 'AC Melita' clustered closely in the AFLP-based, but not in the pedigree-based, dendrogram. These differences may be due to the repeated cycle of recombination and selection during cultivar development which are unaccounted for by pedigree analysis but revealed at the DNA level.

The new or modern cultivars have one feature in common, a relatively high degree of genetic homogeneity (Frankel and Soulé 1981). Among the 13 cultivars in this investigation (Fig. 4), a fairly substantial level of genetic heterogeneity is still maintained in spite of the rigorous selection pressure aimed at cultivar purity, uniformity, and the associated breeding practices directed towards agronomic homogeneity. As can be inferred from Fig. 4 the extent of heterogenity (gene diversity per locus) decreases from right to left from 'Pelissier', an early selection from a landrace, to 'Wascana', a derived cultivar.

Variance components within and between cultivars were measured as 1.72 and 14.67 (Table 4) respectively when all 89 polymorphic markers were used, indicating a much broader range of variation between cultivars compared to the corresponding values within a cultivar. This indicates clearly the results of rigorous selection applied during the breeding process.

The reproducibility of AFLP data, coupled with the high multiplex ratio of this marker system, makes this approach ideal for the studies involving genetic diversity in crop species with a narrow genetic base, such as durum wheat. Furthermore, it also provides a means to fingerprint closely related cultivars for identification, cultivar purity maintenance and for intellectual property protection of newly bred cultivars. Although the pedigree approach remains as an inexpensive way of inferring estimates of genetic relationship in crop plants, molecular markers such as AFLPs are more likely to reflect a true measure of genetic relationships. Unlike pedigree analysis, the AFLP approach can detect the effects of genetic drift and selection, as well as giving a realistic measure of relatedness among ancestors.

Criticism in the use of some DNA markers such as AFLPs for genetic diversity estimates stems from their nature as unmapped markers whose genomic distribution is unclear. Some studies have shown that AFLPs tend to be genetically clustered (Karp et al. 1997) and, as a result, would not represent independent variables (Reeves et al. 1999). But with the relatively large number of markers used in this study, some redundancy resulting from genetic linkage may have been expected; however, this source of error is likely to be insignificant in comparison to the large number of polymorphisms detected and analysed.

In conclusion, there still exists a substantial level of genetic variation within modern cultivars of durum wheat as detected by AFLP, despite rigorous selection pressure aimed at cultivar purity and associated breeding practices. Measurements of genetic diversity can be used in breeding programs in order to maximize the level of variation present in segregating populations by crossing cultivars with greater genetic distance.

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