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REGULAR ARTICLE

Genetic variability in multiple accessions of two Canadian heritage crop cultivars as revealed by AFLP markers

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ABSTRACT

Verifying duplicate accessions of plant germplasm using molecular markers can be challenging, as many of these accessions are genetically heterogeneous. A pilot study was made to quantify the genetic variability in six truly duplicated accessions from each of two Canadian heritage crop cultivars (Marquis wheat and Canadian Thorpe barley). Three amplified fragment length polymorphism (AFLP) primer pairs were applied to genotype 15 seeds for each Marquis accession and 11 seeds from each Canadian Thorpe accession. About 115 polymorphic AFLP bands were scored for each seed sample. Analyses of these AFLP data revealed a wide range of band frequencies from 0.02 to 0.99 with an average of 0.54 for Marquis and 0.52 for Canadian Thorpe. The proportion of the total AFLP variation explained among the six wheat accessions was 5.1% and among the six barley accessions 4.5%. These findings indicate that a threshold value of 5% AFLP variation could be used to verify the duplicates of these heritage cultivars.

Key Words: *genetic variability; wheat; barley; amplified fragment length polymorphism (AFLP); duplicate accession*.

INTRODUCTION

Duplicate accessions of plant germplasm are known to exist in most ex situ collections (Hintum and Knüpffer, 1995; Hintum and Visser, 1995) and redundancy in a collection is a challenging issue in the management and utilization of plant germplasm (Engels and Visser, 2003). Efforts have been made to identify these accessions and many potential duplicate accessions have been compiled, largely relying on passport data (Hintum and Knüpffer, 1995; Hintum and Visser, 1995; Greene and Pederson, 1996). Inadequate attention has been paid to verify the identified accessions, particularly using DNA markers (Waycott and Fort, 1994;

Virk et al., 1995; Phippen et al., 1997; Zeven et al., 1998; Lund et al., 2003). Verifying duplicates of genetically heterogeneous germplasm can be challenging, as a simple matching of DNA profiles between the potential duplicates is not effective (Treuren and Hintum, 2001). Little effort has been made to assess the genetic heterogeneity of truly duplicated accessions (Waycott and Fort, 1994; Arens et al., 1998; Treuren and Hintum, 2001; Lund et al., 2003) and consequently the amount of genetic diversity acceptable between genetically duplicate accessions is not well-defined for duplicate verification (Lund et al., 2003). Concerns have been raised about the accuracy of molecular duplicate verification (Treuren et al., 2001; Lund et al., 2003; Fu, 2006).

We conducted a pilot molecular study on the genetic variability in six truly duplicated accessions from each of two Canadian heritage crop cultivars: Marquis and Canadian Thorpe. Wheat (*Triticum aestivum* L.) cultivar Marquis was developed in 1909 from a cross of Red Fife and Hard Red Calcutta and had played a dominant role for the Canadian wheat industry until its replacement Neepawa was released in 1969 (DePauw et al., 1995). Barley (*Hordeum vulgare* ssp. *vulgare*) cultivar Canadian Thorpe is one of the old-time farmer varieties and probably was a selection from an English barley variety Thorpe or Goldthorpe (Newman and Cowan, 1928). This cultivar was predominantly grown in Manitoba, served as the standard of quality for the top grades of two-row barley in the Canada Grain Act at the turn of the 20th century (Owen and Conacher, 1960). Currently, Plant Gene Resources of Canada (PGRC; the Canadian national seed genebank) at Saskatoon, Saskatchewan, maintains five Marquis and five Canadian Thorpe accessions that were donated by several plant breeders and related agencies from 1955 to 2003. In 2001, PGRC also received non-viable seed samples of Marquis and Canadian Thorpe from Mr. Gill Shaw at Manitoba Agricultural Credit Corporation. These seeds were originated from the 1913 Manitoba Seed Fair, Brandon, Manitoba, and donated by Dr. Seager Wheeler (one of the early dominant wheat breeders), Rosthern, Saskatchewan. Thus, these two cultivars not only are well representative of the early released cultivars, but also have the known identity required for duplication verification.

The objective of this study was to assess genetic variation among six Marquis and six Canadian Thorpe accessions using amplified fragment length polymorphism (AFLP) markers with the hope of developing a threshold value of true duplicates for duplicate verification. The AFLP technique (Vos et al., 1995) has been widely applied in studies of genetic diversity in several crop species (Hill et al., 1996; Zhu et al., 1998; Fu et al., 2004) and has shown its effectiveness in detection of genetic variation.

MATERIALS AND METHODS

PLANT MATERIALS

Germplasm assayed in this study includes five accessions each of Marquis and Canadian Thorpe currently maintained at PGRC and one seed sample each of Marquis and Canadian Thorpe originated from Dr. Seager Wheeler (Table 1). As mentioned above, these two non-viable seed samples were donated by Dr. Seager Wheeler at the 1913 Manitoba Seed Fair, Brandon, Manitoba and provided to PGRC in 2001 by Mr. Gill Shaw from Manitoba Agricultural Credit Corporation through Dr. Reg Kucey, Brandon Research Centre. Fifteen seeds from each Marquis accession and 11 seeds from each Canadian Thorpe accession were randomly sampled for DNA isolation.

DNA EXTRACTION AND AFLP ANALYSIS

Each sampled seed was crushed between pieces of loose leaf paper using pliers, transferred to a 2 mL microcentrifuge tube with five 3 mm glass beads, and ground to a fine powder in a mixer mill. DNA was extracted using a Qiagen (Mississauga, ON, Canada) DNEasy Plant Mini kit (cat. no. 69106) according to the manufacturer's instructions with the exception of extending the initial 65°C incubation with Buffer AP1 to 25 min. Extracted DNA was

quantified in a fluorometer by adding 4 µL of DNA solution to 200 µL of quantification buffer [1 mg L^{-1} Hoechst 33258 dye (Sigma, St. Louis, MO, USA, cat. no. B-2883) in 10 mmol L^{-1} Tris–HCL pH 7.4, 1 mmol L^{-1} EDTA pH 8, 1 mol L^{-1} NaCl] and reading at excitation and emission wavelengths of 355 nm and 450 nm, respectively. These extractions typically yielded 7.5 µg per wheat seed and 2.5 µg per barley seed. Quantified DNA was diluted to 25 ng μL^{-1} for AFLP analysis.

The AFLP™ Analysis System 1 (Invitrogen, Burlington, ON, Canada) was applied following the protocol described by Vos et al. (1995) with exception of labeling with γ33P. Polymerase chain reactions were performed in a PTC-200 DNA Engine thermocycler (MJ Research, Watertown, MA, USA). Three informative EcoRI:MseI primer pairs (E+ACC/M+CAG, E+ACG/M+CTA, and E+ACG/M+CTG) were selected from previous related AFLP analyses and applied to assay both wheat and barley seed samples in this study.

Table 1. Accessions of wheat cultivar Marquis and barley cultivar Canadian Thorpe assayed in this study and observed AFLP patterns.

Cultivar/ accession (code)[a]	Source of donation	Sample size[b]	No. of Polym. bands[c]	Mean band frequency	Within–accession variation[d]
Marquis					
Wheeler (M1)	S.Wheeler, SK (2001)	15	112	0.399	45.98
CN11061 (M2)	G.Boughton, SK (1980)	15	106	0.548	37.01
CN11062 (M3)	G.Boughton, SK (1980)	15	101	0.628	40.54
CN33674 (M4)	W.Pert, ON (1972)	15	107	0.579	45.10
CN45648 (M5)	R.DePauw, SK (1985)	15	103	0.516	44.33
TMP14194 (M6)	D.Gehl, SK (2003)	15	105	0.600	45.01
Canadian Thorpe					
Wheeler (CT1)	S.Wheeler, SK (2001)	11	112	0.459	34.95
CN479 (CT2)	S.Wells, AB (1955)	11	94	0.522	27.85
CN1622 (CT3)	D.Metcalfe, MB (1955)	11	92	0.590	25.53
CN2458 (CT4)	K.Ho, ON (1977)	11	97	0.471	29.82
CN3970 (CT5)	K.May, AB (1955)	11	93	0.544	29.80
CN6831 (CT6)	K.Ho, ON (1981)	11	95	0.542	29.64

[a] Twelve accessions include one Marquis accession and one Canadian Thorpe accession originated from S. Wheeler, SK; one Marquis accession (TMP14194) from D. Gehl at Indian Head, SK; and nine accessions held in Plant Gene Resources of Canada, SK. The sample of each accession was coded either with prefix M for Marquis or CT for Canadian Thorpe.

[b] Source of donation includes the person, the employment or living province, and the donation year.

[c] The number of polymorphic AFLP bands per accession. A total of 115 and 117 polymorphic bands were scored for Marquis and Canadian Thorpe, respectively.

[d] The sum of squares within an accession estimated from AMOVA.

DATA ANALYSIS

For each AFLP gel from a primer pair, the total number of AFLP bands observed was counted, including those monomorphic for all six accessions of a cultivar and for all individual seed samples of single accessions. For those bands polymorphic for all accessions of a cultivar and with clarity, scoring was made with presence (1) or absence (0) for each seed sample. Based on this score matrix, the number of polymorphic bands was determined for each primer pair and accession. The frequency of each scored band occurring in each accession sample was calculated and the average band frequency was estimated for all the poly-

morphic bands scored. The numbers of polymorphic bands were also counted and plotted with respect to their frequencies of occurrence in the seed samples of an accession for each cultivar.

Analysis of molecular variance (AMOVA; Excoffier et al., 1992) was performed using Arlequin version 3.0 (Excoffier et al., 2005) to assess AFLP variations within and among accessions of each cultivar. This analysis not only allows the partition of the total AFLP variation into within- and among- accession variation components, but also provides a measure of genetic distance as the proportion of the total AFLP variation residing between any two accessions of each cultivar commonly called the Phi-statistic (Φst; Excoffier et al., 1992). Significance of the resulting variance components and genetic distances away from zero was tested with 10,100 permutations. To assess the contribution of single accessions to the observed variation, AMOVA was also conducted for the modified data (i.e., without the individual accessions of interest) and the corresponding significance test was made with permutation.

To assess the genetic distinctness of each accession, a principal component analysis was conducted for the individual seed samples of each cultivar using SAS® PROC PRINCOMP (SAS Institute, 2004) treating AFLP data as exploratory variables. The first two resulting principal components were plotted to assess the association of the six accessions and to discern genetically distinct individual seed samples.

RESULTS AND DISCUSSION

AFLP VARIATION

The three AFLP primer pairs used in this study produced a total of 569 AFLP bands for Marquis and 492 bands for Canadian Thorpe. The number of observable bands per primer pair ranged from 172 (for the primer pair E+ACG/M+CTA) to 208 (for the primer pair E+ACC/M+CAG) for Marquis and from 144 (for the primer pair E+ACC/M+CAG) to 175 (for the primer pair E+ACG/M+CTA) for Canadian Thorpe. Only four and one monomorphic bands were observed across the individual seed samples assayed for Marquis and Canadian Thorpe, respectively. The numbers of accession—specific monomorphic bands per primer pair ranged from 5 to 9 for Marquis and from 3 to 6 for Canadian Thorpe. Since a majority of the observed bands lacked clarity, only 115 polymorphic bands that could be unequivocally scored for presence or absence for all individual seed samples of Marquis and 117 polymorphic bands in Canadian Thorpe samples were considered for further analysis. This represented about 38 polymorphic bands scored per primer pair. A wide range of band frequencies was observed from 0.02 to 0.99 with an average of 0.54 for Marquis and from 0.02 to 0.97 with an average of 0.52 for Canadian Thorpe (Figure 1). These results clearly indicate these two cultivars were genetically heterogeneous and far from pure breeding lines.

VARIATION AMONG MARQUIS WHEAT ACCESSIONS

The numbers of polymorphic bands for each Marquis accession ranged from 101 to 112 (Table 1). The five accessions of viable seeds (M2–M6) revealed compatible AFLP variations, except for the accession of non-viable seeds (M1). The non-viable seeds displayed more polymorphic bands and lower average band frequency than the viable seeds. Such difference can also be seen in Figure 1A, in which a large proportion of polymorphic bands for M1 had band frequencies of 0.3 to 0.4. This may reflect the impact of deteriorated DNA extracted from non-viable seeds. The non-viable seeds displayed lower DNA yields per seed and fewer bands of high molecular weight than the viable seeds. Despite this, the within-accession variation for these non-viable seed samples estimated from AMOVA was similar to those for the other Marquis accessions, although the accession M2 tended to have the lowest within-accession variation (Table 1).

Partitioning of the total AFLP variation into within- and among- accession components by AMOVA showed 94.9% of the total variation was present within single accessions and

only 5.1% among the six accessions (Table 2). The among-accession variation, although small, was significant from zero ($P < 0.0001$) based on the permutation test. The accession of non-viable seeds contributed the most variation observed among the six accessions. Removing these non-viable seed samples resulted in only 1.8% of the total AFLP variation being explained among the other five accessions, although still significantly different from zero ($P < 0.0032$). Further removing of the accession M5 revealed 1.4% of the total AFLP variation being explained among the four remaining accessions (M2, M3, M4, and M6) with a significance level of $P < 0.0274$. Assessments of inter-accession distances obtained among these six Marquis accessions (Table 3) revealed significant deviations of the accessions M1 and M5 occurring against the other four accessions. Despite these differences, the biplot of the first two principal components for 89 individual seed samples revealed little grouping among these six Marquis accessions (Figure 2A).

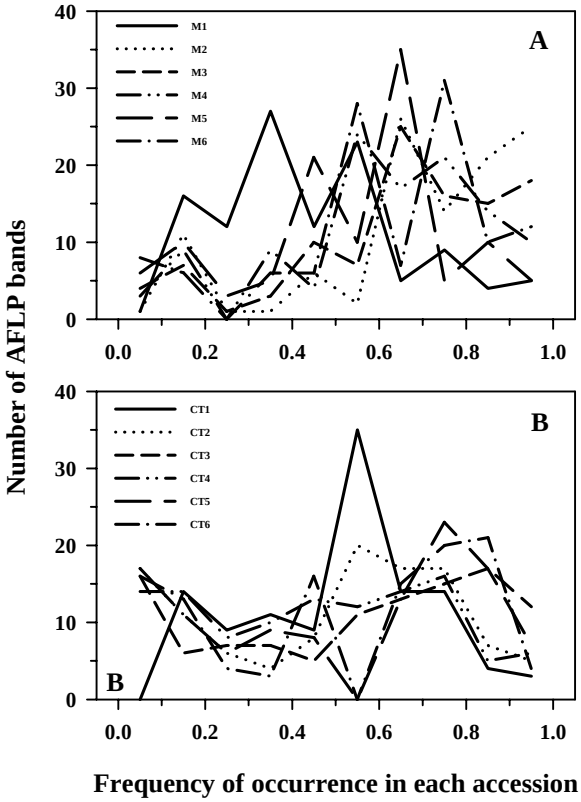

Figure 1. Numbers of AFLP bands with respect to their frequencies of occurrence in each accession of wheat cultivar Marquis (**A**) and barley cultivar Canadian Thorpe (**B**).

VARIATION AMONG CANADIAN THORPE BARLEY ACCESSIONS

The numbers of polymorphic bands for each Canadian Thorpe accession ranged from 92 to 112 (Table 1). The five accessions of viable seeds (CT2–CT6) revealed similar AFLP variations, but the non-viable seeds (CT1) displayed more polymorphic bands and lower average band frequency than the viable seeds.

Such difference was clearly illustrated in Figure 1B, in which there were a larger proportion of polymorphic bands displaying band frequencies of 0.5 to 0.6 and fewer bands of frequencies less than 0.1 for CT1 than the other accessions.

Table 2. Results for analysis of molecular variance for 89 seeds representing six accessions of wheat cultivar Marquis based on 115 AFLP markers and for 64 seeds representing six accessions of barley cultivar Canadian Thorpe based on 117 AFLP markers.

Cultivar/source	*df*[a]	Sum of squares	Variance component	Percentage of variation	*P*-value[b]
Marquis					
Among samples	5	192.87	1.15	5.08	<0.0001
Within samples	83	1785.43	21.51	94.92	
Total	88	1978.30	22.66		
Canadian Thorpe					
Among samples	5	111.11	0.70	4.49	<0.002
Within samples	58	858.24	14.80	95.51	
Total	63	969.34	15.49		

[a] One individual wheat seed sample and two individual barley seed samples with too many missing AFLP data were removed from the analysis.
[b] The probability that the among-sample variance component was larger than zero, as computed from 10,100 permutations.

Table 3. Inter-accession distances among six accessions of wheat cultivar Marquis and among six accessions of barley cultivar Canadian Thorpe, as estimated from analysis of molecular variance (AMOVA).

Accession pair of Marquis	Inter-accession distance, Φst	*P*-value[a]	Accession pair of Canadian Thorpe	Inter-accession distance, Φst	*P*-value
M1 vs M2	0.180	<0.0001	CT1 vs CT2	0.030	
M1 vs M3	0.144	<0.0001	CT1 vs CT3	0.075	0.0112
M1 vs M4	0.083	<0.0001	CT1 vs CT4	0.044	
M1 vs M5	0.039	0.0039	CT1 vs CT5	0.035	
M1 vs M6	0.083	0.0014	CT1 vs CT6	0.037	
M2 vs M3	0.010		CT2 vs CT3	0.121	0.0025
M2 vs M4	0.009		CT2 vs CT4	0.016	
M2 vs M5	0.062	0.0001	CT2 vs CT5	0.034	
M2 vs M6	0.034	0.0219	CT2 vs CT6	0.023	
M3 vs M4	0.014		CT3 vs CT4	0.082	0.0096
M3 vs M5	0.040	0.0010	CT3 vs CT5	0.076	0.0172
M3 vs M6	0.025	0.0436	CT3 vs CT6	0.094	0.0070
M4 vs M5	0.002		CT4 vs CT5	0.000	
M4 vs M6	0.000		CT4 vs CT6	0.003	
M5 vs M6	0.000		CT5 vs CT6	0.000	

[a] The probability of a random Φst value was greater than the observed value, as estimated from 10,100 permutations in AMOVA; only $P < 0.05$ values are shown.

Thus, the largest within-accession variation was observed for the accession of non-viable seeds. It remains unknown, however, whether such difference was mainly due to the impact of the deteriorated DNA extracted from non-viable seeds.

Partitioning of the total AFLP variation into within- and among- accession components by AMOVA showed 95.5% of the total variation was present within single accessions and only 4.5% among the six accessions (Table 2). The among-accession variation, although small, was significantly different from zero ($P < 0.002$) based on the permutation test. The accession of non-viable seeds, although with large within-accession variation, did not contribute much to the variation observed among the Canadian Thorpe accessions. However, removing the accession CT3 generated a lower, non-significant ($P = 0.0641$) proportion (2.2%) of the total AFLP variation being explained among the five remaining accessions. Assessments of inter-accession distances obtained among these six Canadian Thorpe accessions (Table 3) revealed significant deviations of the accession CT3 from the other five accessions. Examinations of the biplot of the first two principal components for 64 individual seed samples revealed little grouping among these six Canadian Thorpe accessions, although some non-viable seeds appear to be somewhat distant from the other seeds (Figure 2B).

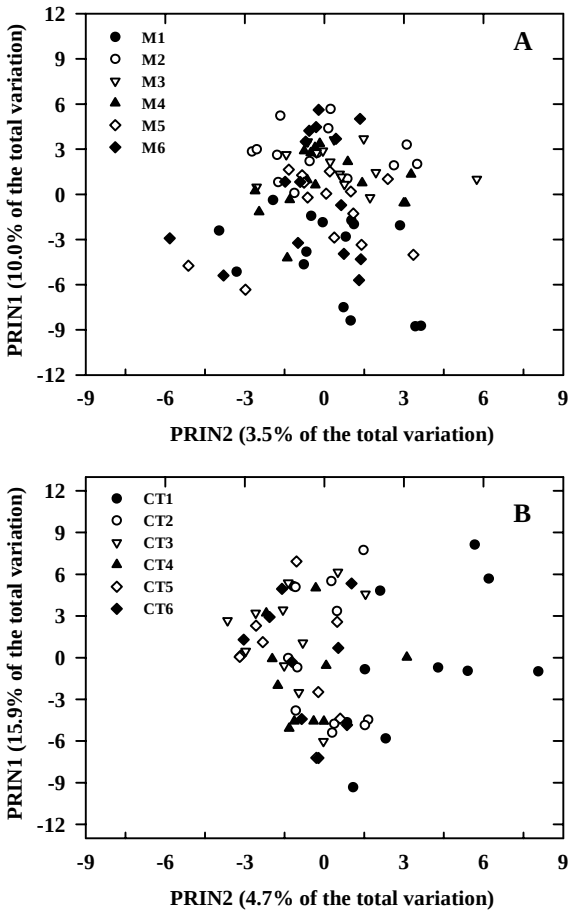

Figure 2. Associations of 89 seeds of wheat cultivar Marquis and 64 seeds of barley cultivar Canadian Thorpe in the biplots of the first two principal components (PRIN1 versus PRIN2) which were estimated from the principal component analyses of their original AFLP data. **A**: The biplot for Marquis in which seeds of six accessions are separately labeled with a prefix M. **B**: The biplot for Canadian Thorpe in which seeds of six accessions are separately labeled with a prefix CT.

IMPLICATIONS FOR PLANT GERMPLASM MANAGEMENT

The AFLP analysis presented here revealed more genetic heterogeneity within these heritage cultivars than previously thought; such heterogeneity is not expected from a pure breeding line. Only small variation (< 5%) was observed among the accessions of each cultivar. Such a level of inter-accession variation could be partly explained by the impact of the deteriorated DNA of non-viable seeds and/or the use of small sample size, but probably not due to genetic drift and shift from rejuvenating these accessions before being entered to the PGRC collection. Even with only a few cycles of rejuvenation, much large variation is expected (Parzies et al., 2000; Börner et al., 2000). Apparently, more seeds should be assayed for each accession to make such an AFLP assessment more informative.

The findings presented here are significant for duplicate verification. True duplicates of the two heritage cultivars can display up to 5% AFLP variation among duplicates, which implies that the molecular verification of true duplicates could still vary in accuracy. The value of 5% obtained here is higher than those reported for identifying genetically identical accessions [2% RAPD variation from Waycott and Fort (1994); 2% AFLP variation from Arens et al. (1998)], but is at the lower end of those used to determine genetically redundant accessions [4.6% RAPD variation from Phippen et al. (1997); 5% RAPD from Fu (2006); 10% SSR variation from Dean et al. (1999); 14% SSR variation from Lund et al. (2003)]. Thus, a threshold value of 5% AFLP variation could be used to verify the duplicates of these heritage cultivars. Such a value might also be useful as a guide for future duplicate verification of genetically heterogeneous germplasm, particularly those of selfing crop plants. However, given an ex situ collections generally is composed of various types of plant germplams (Treuren and Hintum, 2001), specific heterogeneity assessments on some sets of true duplicate from a collection should be made for more accuracy of duplicate verification.

The findings of this study also have implications for plant germplasm management. First, a management action could be taken to minimize the verified duplicates, but at least one safety duplicate should be considered for important heritage cultivars such as Marquis. Second, as the earlier developed cultivars are genetically heterogeneous, it also is a challenge to maintain cultivar identity in further rejuvenation of these and other heritage cultivars. Third, this pilot study demonstrated that the AFLP technique is a useful tool for duplication validation. Applications to future duplicate verification should be encouraged.

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REFERENCES

Arens, P., Coops, H., Jansen, J., Vosman, B. (1998). Molecular genetic analysis of black poplar (*Populus nigra* L.) along Dutch rivers. *Mol. Ecol. 7*, 11–18.

Börner, A., Chebotar, S., Korzun, V. (2000). Molecular characterization of the genetic integrity of wheat (*Triticum aestivum* L.) germplasm after long-term maintenance. *Theor. Appl. Genet.* 100, 494–497.

Dean, R.E., Dahlberg, J.A., Hophins, M.S., Mitchell, S.E., Kresovich, S. (1999). Genetic redundancy and diversity among 'Orange' accessions in the U.S. national sorghum collection as assessed with simple sequence repeat (SSR) markers. *Crop Sci.* 39, 1215–1221.

DePauw, R.M., Boughton, G.R., Knott, D.R. (1995). Hard red spring wheat. In: Slinkard, A.E., Knott, D.R. (Eds.): *Harvest of Gold: the History of Field Crop Breeding in Canada*. University of Saskatchewan, SK, Canada, 5–35.

Engels, J.M.M., Visser, L. (2003). *A guide to effective management of germplasm collections*. IPGRI handbooks for genebanks No. 6, IPGRI, Rome, Italy.

Excoffier, L., Smouse, P.E., Quattro, J.M. (1992). Analysis of molecular variance inferred from metric distances among DNA haplotypes: Application to human mitochondrial DNA restriction data. *Genetics* 131, 479–491.

Excoffier, L., Laval, G., Schneider, S. (2005). Arlequin ver. 3.0: An integrated software package for population genetics data analysis. *Evol. Bioinformatics Online* 1, 47–50.

Fu, Y.B. (2006). Redundancy and distinctness in flax germplasm as revealed by RAPD dissimilarity. *Plant Genet. Resour.* (in press).

Fu, Y.B., Kibite, S., Richards, K.W. (2004). AFLP analysis of 96 Canadian oat cultivars released from 1886 to 2001. *Can. J. Plant Sci.* 84, 23–30.

Greene, S.L., Pederson, G.A. (1996). Eliminating duplicates in germplasm collections: a white clover example. *Crop Sci.* 36, 1398–1400.

Hill, M., Witsenboer, H., Zabeau, M., Vos, P., Kesseli, R., Michelmore R. (1996). PCR-based fingerprinting using AFLPs as a tool for studying genetic relationship in *Lactuca* spp. *Theor. Appl. Genet.* 93, 1202–1210.

Hintum, Th.J.L. van, Knüpffer, H. (1995). Duplication within and between germplasm collections. I. Identifying duplication and the basis of passport data. *Genet. Resour. Crop Evol.* 42, 127–133.

Hintum, Th.J.L. van, Visser, D.L. (1995). Duplication within and between germplasm collections. II. Duplication in four European barley collections. *Genet. Resour. Crop Evol.* 42, 135–145.

Lund, B., Ortiz, R., Skovgaard, I.M., Waugh, R., Andersen, S.B. (2003). Analysis of potential duplicates in barley gene bank collections using re-sampling of microsatellite data. *Theor. Appl. Genet.* 106, 1129–1138.

Newman, L.H., Cowan, P.R. (1928). *Barley culture in Canada*. Dominion of Canada Department of Agriculture pamphlet No. 99 – new series, pp. 47.

Owen, C.H., Conacher, M.J. (1960). *Identification of barley and wheat varieties by kernel characters*. Board of Grain Commissioners for Canada, Inspection Branch, Winnipeg, Manitoba, pp. 107.

Parzies, H.K., Spoor, W., Ennos, R.A. (2000). Genetic diversity of barley landrace accessions (*Hordeum vulgare* ssp. *vulgare*) conserved for different lengths of time in ex situ gene banks. *Heredity* 84, 476–486.

Phippen, W.B., Kresovich, S., Candelas, F.G., McFerson, J.R. (1997). Molecular characterization can quantify and partition variation among genebank holdings: a case study with phenotypically similar accessions of *Brassica oleracea* var. *capitata* L. (*cabbage*) "Golden Acre". *Theor. Appl. Genet.* 94, 227–234.

SAS Institute Inc. (2004) *The SAS system for windows V8.02*. SAS Institute Incorporated, Cary, NC, USA.

Treuren, R. van, Hintum, Th.J.L. van (2001). Identification of intra-accession genetic diversity in selfing crops using AFLP markers: implications for collection management. *Genet. Resour. Crop Evol.* 48, 287–295.

Treuren, R. van, Soest, L.J.M. van, Hintum, Th.J.L. van (2001). Marker-assisted rationalization of genetic resources collections: a case study in flax using AFLPs. *Theor. Appl. Genet.* 103, 144–152.

Virk, P.S., Newbury, H.J., Jackson, M.T., Ford-Lloyd, B.V. (1995). The identification of duplicate accessions with a rice germplasm collection using RAPD analysis. *Theor. Appl. Genet.* 90, 1049–1055.

Vos, P., Hogers, R., Bleeker, M., van Reijans, M., De Lee, T., Hornes, M., Frijters, A., Peleman, J., Kuiper, M., Zabeau, M. (1995). AFLP: a new technique for DNA fingerprinting. *Nucleic Acids Res.* 23, 4407–4414.

Waycott, W., Fort, S.B. (1994). Differentiation of nearly identical germplasm accessions by a combination of molecular and morphological analyses. *Genome* 37, 577–583.

Zeven, A.C., Dehmer, J.K., Gladis, T., Hammer, K., Lux, H. (1998). Are the duplicates of perennial kale (*Brassica oleracea* L. var. *ramosa* DC.) true duplicates as determined by RAPD analysis? *Genet. Resour. Crop Evol.* 45, 105–111.

Zhu, J., Gale, M.D., Quarrie, S., Jackson, M.T., Bryan, G.I. (1998). AFLP markers for the study of rice biodiversity. *Theor. Appl. Genet.* 96, 602–611.