Quantitative Evaluation of Genetic Diversity in Wheat Germplasm Using Molecular Markers

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ABSTRACT

Characterization of germplasm by means of DNA fingerprinting techniques provides a tool for precise germplasm identification and a quantitative estimate of genetic diversity. This estimate is important because a decrease in genetic variability might result in a reduction of the plasticity of the crops to respond to changes in climate, pathogen populations, or agricultural practices. In this study, 105 Argentine bread wheat (*Triticum aestivum* L.) cultivars released between 1932 and 1995 were characterized by simple sequence repeat (SSR) and amplified fragment length polymorphism (AFLP) markers. A selected subset of 10 highly informative SSR was used to construct an Identification Matrix that allowed the discrimination of the 105 cultivars. Data obtained from SSR markers were complemented by information derived from AFLPs. Molecular data were used to quantify genetic diversity across Argentine wheat breeding programs and to determine if modern wheat cultivars have a lower genetic diversity than earlier cultivars (genetic erosion). No significant differences in genetic diversity were found among the large private and public breeding programs, suggesting that each of them contains a representative sample of the complete diversity of the Argentine germplasm. Significant differences were found for both SSR and AFLP only between breeding programs with large differences in number of released cultivars. No significant differences in genetic diversity were found between the group of cultivars released before 1960 and those released in each of the following three decades. Average diversity values based on SSR markers were almost identical for the four analyzed periods. Genetic diversity estimates based on AFLP data confirmed the absence of a reduction of genetic diversity with time, but significant differences ($P = 0.01$) were found between bread wheat cultivars released in the 1970s (PIC = 0.28) and those released in the 1980s (PIC = 0.34). These results show that the Argentine bread wheat germplasm has maintained a relatively constant level of genetic diversity during the last half century.

IDENTIFICATION AND REGISTRATION of bread wheat cultivars is mainly based on morphologic and physiologic characteristics. Even though these descriptors are useful, they are limited in number and may be affected by environmental factors. Molecular markers are a useful complement to morphological and physiological characterization of cultivars because they are plentiful, independent of tissue or environmental effects, and allow cultivar identification early in plant development. Molecular characterization of cultivars is also useful to evaluate potential genetic erosion, defined here as a reduction of genetic diversity in time.

Restriction fragment length polymorphism (RFLP, Bostein et al., 1980) was one of the first DNA marker techniques used to characterize wheat cultivars (Vaccino et al., 1993) and assess genetic diversity (Kim and Ward, 1997; Paull et al., 1998). However, the relatively low level of polymorphism observed among elite wheat cultivars (Bryan et al., 1999) and the complexity and cost of the technique, limit the use of RFLP for routine cultivar identification. The polymerase chain reaction (PCR) technique facilitated the development of a second generation of simpler and lower-cost molecular markers, including SSR (also known as microsatellites, Tautz and Renz, 1984) and AFLP (Vos et al., 1995).

The SSR technique gained rapid acceptability because of its codominant nature, reproducibility, and high information content (De Loose and Gheysen, 1995). These loci are amplified by PCR using primers (18–25 bp long) specific for sequences flanking hypervariable regions of tandem repeats of 2 to 4 base pairs. The variation in the number of repeats present in these loci determines differences in length of the amplified fragments. This methodology is useful in identifying genotypes in self-pollinated species with low levels of genetic variability such as soybean [*Glycine max* (L.) Merr.] (Rongwen et al., 1995), rice (*Oryza sativa* L.) (Yang et al., 1994), and wheat (Domini et al., 2000).

In wheat, two independent studies showed that SSR provide a greater level of intraspecific polymorphism than RFLP (Röder et al., 1995, Plaschke et al., 1995) and prompted the development of more than 400 SSR loci in wheat (Röder et al., 1995; Devos et al., 1995; Plaschke et al., 1996; Bryan et al., 1997; Röder et al., 1998; Stephenson et al., 1998). The first SSR markers available were used to characterize eight European cultivars (Devos et al., 1995) and 11 Canadian cultivars (Lee et al., 1995) of wheat bread. In a more comprehensive study of 40 European bread wheat cultivars using 23 SSR, Plaschke et al. (1995) concluded that a relative small number of SSR was sufficient to discriminate this set of cultivars.

The AFLP technique combines the RFLP reliability with the power of PCR to amplify simultaneously many restriction fragments (Vos et al., 1995). This technique was used successfully to evaluate genetic diversity and genetic relationships in wheat (Salamini et al., 1997; Barrett and Kidwell, 1998; Domini et al., 2000), bean (*Phaseolus vulgaris* L.) (Tohme et al., 1996), rice (Mackill et al., 1996; Virk et al., 2000), tea (*Camellia sinensis* Kuntze) (Paul et al., 1997), barley (*Hordeum vulgare* L.) (Qi and Lindhout, 1997), and soybean (Maughan et al., 1996).

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Abbreviations: AFLP, amplified fragment length polymorphism; bp, base pairs; GS, genetic similarity; *f*, kinship coefficient; PIC, Polymorphism Index Content; *r*, correlation coefficient; RFLP, restriction fragment length polymorphism; SSR, simple sequence repeat or microsatellite; UPGMA, unweighted pair-group method with arithmetic averages.

In this manuscript, we present the characterization of 105 bread wheat cultivars from Argentina using SSRs and AFLP markers. The first objective of this work was to develop an Identification Matrix to facilitate a rapid and accurate identification of the Argentine bread wheat germplasm. The second objective was to quantify the effect of a half century of wheat breeding on genetic diversity and to evaluate potential genetic erosion. Finally, we wanted to understand the contribution of different public and private breeding programs to the total genetic diversity of the Argentine gene pool.

MATERIALS AND METHODS

Plant Material and DNA Isolation

This study included 105 bread wheat cultivars from Argentina registered in the National Seed Property Register of Argentina (INASE) and released between 1932 and 1995. The complete list of cultivars with their origin, date of release, and SSR alleles is available at Appendix 1 located in http://agronomy.ucdavis.edu/Dubcovsky/Argentina/SSR_Argentina.htm (verified November 30, 2000).

DNA was extracted from a bulk of leaves from five plants from each cultivar by the method described by Maroof et al. (1984). The cultivars used in this study are homozygous lines, but five plants per cultivar were pooled for DNA extraction to avoid the possibility of selecting a single contaminating seed. Gilbert et al. (1999) also recommended the use of pools from five plants to assess genetic variability with DNA markers in large plant germplasm collections.

PCR Markers

SSR loci used in this study were developed by Devos et al. (1995) (*Xpsp*, Table 1), Röder et al. (1995) (*Xgwm*, Table 1), and Ma et al. (1996) (*Xcnl*, Table 1). Primer sequences and annealing temperatures are included in Table 1.

Amplification reactions were carried out in a Perkin Elmer thermocycler model 480 (Perkin-Elmer, Norwalk, CT) in a 12-μL reaction mixture. Each reaction contained 200 μ*M* dNTPs, 1.5 m*M* Mg^{++} (except for SSR *Xcnl3*, for which 3 m*M* Mg^{++} was used), 100 n*M* of each primer, 0.5 U of *Taq*-polymerase, and 25 ng of genomic DNA as template. Amplification products were separated on 6% (w/v) polyacrylamide denaturing gels and were detected by silver staining (Silver Sequence Promega Biotech, Madison, WI). Size of each band was estimated simultaneously by means of a 25-bp DNA Ladder (Life Technologies-Gibco BRL) and a sequencing reaction for M13 ssDNA as molecular weight markers in adjacent lines of the gel. Amplified SSR fragments of different size were considered as different alleles (Table 1).

AFLP assays were performed as described in Kahn et al. (2000). Briefly, 500 ng of wheat genomic DNA were subject to restriction-ligation in a single step during 6 h in a 30-μL reaction mix (10 m*M* Tris-acetate pH 7.5, 10 m*M* MgAc, 50 m*M* KAc, 5 m*M* DTT, 50 ng/mL BSA, *Pst*I (5U), *Mse*I (5U), and T4 DNA ligase 1U, 5 pmol *Pst*I adaptors, 50 pmol *Mse*I adaptors, and 12 pmol ATP). Five microilters of each adaptor-ligated template DNA were preamplified in a 25-μL PCR reaction containing 75 ng of both P01 and M01 AFLP primers (5′-GAC TGC GTA CAT GCA GA-3′ and 5′-GAT GAG TCC TGA GTA AA-3′, respectively), 0.2 m*M* dNTPs, 1× PCR buffer (1.5 m*M* $MgCl_2$) and 1U of Amplitaq LD (Perkin Elmer). Selective amplifications were performed with 1 μL of nondiluted preamplification product and 30 ng of each selective nonlabeled +3 primer using the cycling conditions described by Vos et al. (1995). Ten microliters of formamide dye were added to the 20-μL PCR reactions and amplification products were separated in 6% (w/v) denaturing polyacrylamide gels and stained by the same procedure as for the SSR gels. The four selective primer combinations assayed were P36 = ACC and M44 = ATC, P40 = AGC and M39 = AGA, P31 = AAA and M41 = AGG, and finally P41 = AGG and M40 = AGC.

Data Analysis. Variability Estimation

Variability for each locus was measured using the Polymorphism Index Content (PIC) (Anderson et al., 1993)

$$PIC = 1 - \sum_{i}^{n} p_i^2$$

where p_i is the frequency of the ith allele.

Table 1. Description of the SSR loci used to develop the Identification Matrix including their chromosome location, primer sequence, annealing temperature, size range of the amplified fragments, number of alleles, and polymorphism index content (PIC) of each SSR.

Locus	Chrom.	Primers	Annual temperature °C	Size range (bp) pairs	No. of Alleles	PIC
Xpsp2999	1AS	TCC CGC CAT GAG TCA ATC TTG GGA GAC ACA TTG GCC	55°C	133–157	10 + 1 null*	0.84
Xpsp3000	1BS	GCA GAC CTG TGT CAT TGG TC GAT ATA GTG GCA GCA GGA TAC	55°C	213–285	12 + 1 null*	0.84
Xcn15	1AL	GGT GAT GAG TGG CAC AGG CCC AAC AGT TGC AGA AAA TTA G	60°C	115–129	5	0.40
Xcn13	6BS	AGA ACA GTC TTC TAG GTT AG CGA GGG ACA GAC GAA TC	50°C	117–151	9 + 1 null	0.63
Xgwm46	7BS	GCA CGT GAA TGG ATT GGA C TGA CCC AAT AGT GGT CA	60°C	159–187	11	0.76
Xgwm44	7DS	GTT GAG CTT TTC AGT TCG GC ACT GGC ATC CAC TGA GCT G	60°C	160–180	9 + 1 null	0.85
Xgwm2	2AS	CTG CAA GCC TGT GAT CAA CT CAT TCT CAA ATC GAA CA	50°C	140–164	7 + 1 null	0.73
Xgwm18	4BS	TGG CGC CAT GAT TGC ATT ATC ATC TTC GGT TGC TGA AGA ACC TTA TTT AGG	50°C	184–196	5	0.67
Xgwm33	1A, 1B, 1D	GGA GTC ACA CTT GTT TGT GCA CAC TGC ACA CCT AAC TAC GTG C	60→46°C‡	138–206	12 + 1 null	0.81
Xgwm5	3AS	GCC AGC TAC CTC GAT ACA ACT C AGA AAG GGC CAG GCT AGT AGT	50°C	167–173	8	0.70

* Null alleles in *Xpsp2999* and *Xpsp3000* correspond to cultivars with the 1AL/1RS and 1BL/1RS rye translocations, respectively (Bullrich et al., 1996).
‡ Touch down from 60°C to 46°C, decreasing 1°C per Cycle, and 30 cycles at 46°C.

For the AFLP analysis, each polymorphic fragment was scored as a locus with two allelic classes. Absolute PIC values of SSR and AFLP markers are not comparable because the maximum PIC value of an AFLP locus is 0.5. However, comparisons between genetic diversity values for different groups of cultivars (breeding programs, decades, etc.) within each marker class are valid. The AFLP analysis was included here to validate the patterns of genetic diversity observed by means of the SSR markers using an independent set of molecular markers.

Genetic diversity was estimated as a measure of genetic variation by the formula of Weir (1996),

$$D = 1 - \frac{1}{L}\sum_{l}\sum_{i} p_i^2$$

where p is the frequency of the i allele at the l locus and L is the number of loci. This formula is equivalent to an average PIC value.

Differences in genetic diversity between decades and breeding programs were evaluated by two-way analysis of variance. PIC values were calculated for each locus for the cultivars grouped in each breeding program or released during each decade. Loci were used as blocks to separate the variation among loci from the error term and increase the sensitivity of the statistical analysis. Normality, additivity, and homogeneity of variances were tested by Shapiro-Wilk, Tukey, and Levene's tests, respectively (SAS Institute, 1994). Variance heterogeneity in the ANOVA among breeding programs was corrected by a $\mathrm{LOG}_{10}(X + 1)$ transformation (average genetic diversity values presented on Table 3 are on the original scale).

Genetic Relationships

Presence or absence of each single fragment was coded as one or zero, respectively, in a binary data matrix for both SSR and AFLPs. Dice's coefficient (Sneath and Sokal, 1973) was selected to construct the similarity matrix. Cluster analysis was performed by the UPGMA method and the NTSYS pc v. 1.8 computer program (Rohlf, 1989).

Kinship coefficient (f) was calculated by means of a linear algorithm and following the assumptions of Cox et al. (1986). Accurate pedigree records were available only for 82 cultivars. Calculations were made on the basis of parentage information extracted from CIMMYT database by the IWIS program, version 1 (Fox et al., 1996).

Correlations between similarity matrices derived from SSR, AFLP, and kinship coefficients were calculated by Pearson product-moment and the significance of the correlation was tested by Mantel's test (Mantel, 1967) with the NTSYS program (MXCOMP module). A second correlation was calculated between similarity matrices derived from kinship coefficients and molecular markers with pairs of cultivars that had kinship coefficients >0.10, following the recommendation of Plaschke et al. (1995).

All statistical analyses were performed by SAS programs (SAS Institute, 1994). Throughout the text, variation measures indicated after the means are the standard deviations of the distributions.

RESULTS

SSR

A subset of 15 bread wheat cultivars was randomly selected from the 105 registered cultivars and was screened with 33 pairs of SSR primers. From this set of 33, 10 SSR were selected for the Identification Matrix on the basis of a combination of the following criteria: (i) high PIC values (>0.60), (ii) repeatability and clarity of the banding pattern (Fig. 1 shows an example of a clear pattern), (iii) absence of close linkage to any other locus previously included in the matrix, and (iv) ability to separate cultivars not differentiated by other SSRs. For example, SSR locus *Xcnl5* with a PIC value <0.60 was included because of its contribution to differentiate pairs of cultivars that could not be differentiated with other SSR of higher PIC value. Correlations between pairs of distance matrices that were calculated for individual SSR loci were not significant indicating that the SSR information is non-redundant. Correlation values varied from 0.128 (between *Xwmg5* and *Xwmg2* loci) to −0.036 (between *Xwmg33* and *Xwmg46* loci) and no significant differences were found between the few loci located in the same chromosome compared to those located in different chromosomes. The number of alleles per locus ranged from 5 to 13 with an average of 9.4, and the PIC values ranged from 0.40 to 0.84 with an average value of 0.72 ± 0.14 (Table 1).

The average similarity coefficient among cultivars showed a normal distribution with an average of 0.29 ± 0.14. Similarity coefficient ranged from 0.90 for closely related pairs of cultivars (Prointa Bonaerense Redomón and Klein Dorado, Diamante INTA and Leones INTA, and Ciano and Norkin T82) to zero for 116 pairs of cultivars. This set of SSR markers was sufficient to differentiate unequivocally all cultivars including the two pairs of sister lines Don Ernesto INTA and Cooperación Calquín and Prointa Pigüé and Prointa Querandí. The complete identification matrix for the 105 registered bread wheat cultivars on the basis of these 10 SSR loci is available at http://agronomy.ucdavis.edu/Dubcovsky/Argentina/SSR_Argentina.htm; verified January 11, 2001.

AFLP

The four pairs of primers described in Materials and Methods were used to generate AFLP fingerprintings. The number of polymorphic bands range from 9 to 27 with an average of 17.8 ± 7.6 per primer combination. PIC values ranged from 0.26 to 0.38 with an average value of 0.30 ± 0.15.

The average similarity coefficient among cultivars based on 71 polymorphic AFLP alleles showed a normal distribution with and average of 0.55 ± 0.10. Similarity coefficients ranged from 0.91 ('Victoria INTA' and 'Saira INTA') to 0.24 ('Prointa Pincén' and 'Prointa Bonaerense Redomón').

Genetic Relationships

Individual dendrograms based on SSR (105 cultivars) and AFLP (96 cultivars) are available at http://agronomy.ucdavis.edu/Dubcovsky/Argentina/SSR_Argentina.htm. Nine DNA samples were degraded and could not be used in the AFLP study. A dendrogram based on combined SSR and AFLP data for the 67 bread wheat cultivars, for which both types of molecular markers and pedigree data were available, is presented in Fig. 2 (38 cultivars with unknown pedigree information were excluded from this analysis).

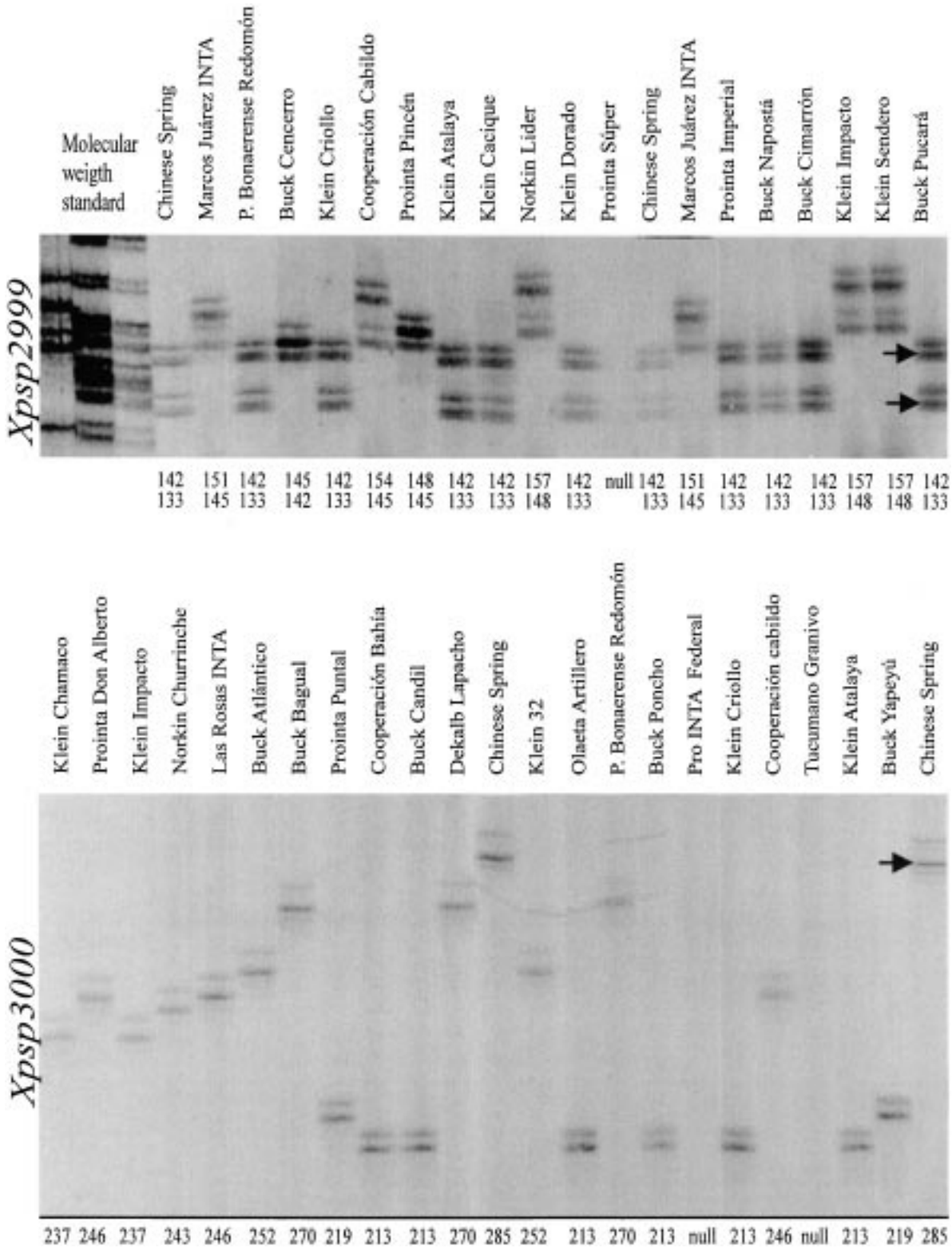

Fig. 1. Bread wheat genotypes assayed with SSR locus *Xpsp2999* = locus *Glu-A3* (1AS) and *Xpsp3000* = *Gli-B1* (1BS). *Xpsp2999* amplified a single major band and *Xpsp3000* two (see arrows). Each main band has "stutter" fainter bands separated by 3 bp produced by errors of the polymerase during the amplification of these trinucleotide SSRs. The size of the main bands in base pairs is detailed at the bottom of the figure.

No clear clustering of cultivars by breeding program or year of release was observed in any of the dendrograms. However, cultivars that belong to the same cluster group generally share common ancestors. Available pedigree information was validated in most cases by the SSR data. For example, the 252-bp allele present in 'Klein 32' at *Xpsp3000*, and associated with good breadmaking quality (Manifesto et al., 1998), was inherited

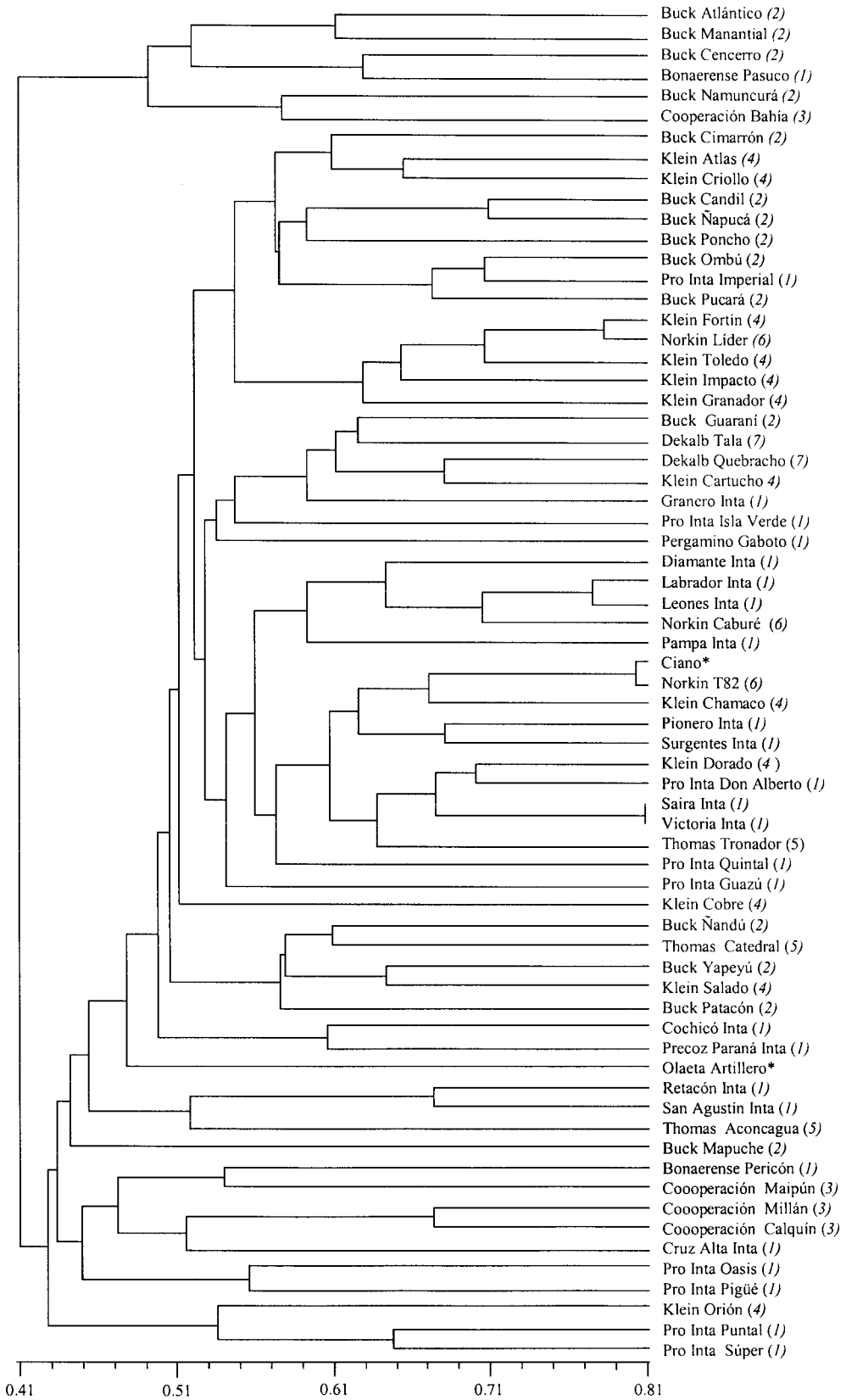

Fig. 2. Dendrogram of 67 bread wheat cultivars from Argentina based on 10 SSR and 71 AFLP fragments. Only cultivars with known pedigree were included in this dendrogram. Values in the *X*-axis correspond to Dice coefficients of similarity. Numbers in italics between brackets indicate breeding programs: (*1*) Inta, (*2*) Buck, (*3*) ACA, (*4*) Klein, (*5*) Thomas, (*6*) Northrup King, and (*7*) Dekalb.* Cultivar 'Ciano' was released by CIMMYT and 'Olaeta Artillero' by I. Vigliano (these two cultivars were not included in the analysis of Table 3).

Table 2. Correlation (r) between similarity matrices based on molecular markers and kinship coefficients.

	SSR	AFLP	SSR + AFLP
Kinship coefficient matrix	0.26**	0.30**	0.34**
Kinship coefficients > 0.10	0.18**	0.34**	0.34**
SSR	–	0.27**	–

** Mantel test, $P < 0.01$.

from 'Americano 25e' through 'Buck Quequén', 'General Urquiza', and 'San Martín'. The 252-bp allele also is present in other cultivars that share some of these ancestors ('Klein Granador', 'Buck Manantial', 'Buck Cencerro', 'Buck Atlántico', 'Buck Cimarrón', 'Buck Napostá', 'Buck Pangaré', and 'Oncativo INTA') but is absent in the rest of the germplasm.

Correlation between Similarity Matrices

Kinship coefficients were calculated for the 67 cultivars with available pedigree information. Kinship coefficients had a maximum value of $f = 0.64$ and a minimum of $f = 0.00$, with an average value of $f = 0.08 \pm 0.13$. Sixty-three percent of the pairs of cultivars analyzed in this study showed kinship coefficient lower than 0.10.

Two correlations coefficients based on different subsets of cultivar pairs were calculated between f and SSR similarity matrices, and between f and AFLP similarity matrices. The first correlation was based on all 2211 pairs of cultivars and the second one on the 690 pairs of cultivars with $f > 0.10$ (Table 2). All correlations were statistically significant (Mantel test, $P < 0.01$). The correlation between the SSR and AFLP similarity matrices also was low ($r = 0.27$) but highly significant (Mantel test, $P < 0.001$).

Variation of Genetic Diversity among Breeding Programs

A two-way analysis of variance using loci and breeding programs as independent variables detected significant differences in genetic diversity among sets of cultivars from different wheat breeding programs in both SSR and AFLP data (Table 3). However, significant differences were found only between the breeding programs that have released a large number of cultivars (INTA = 28 released cultivars and Buck = 18 released cultivars) and the small breeding programs that have released five or fewers cultivars ('Dekalb', 'Northrup King', and 'Thomas'). This was expected because small subsets of cultivars tend to have many loci with PIC values of zero.

A highly significant correlation was detected between genetic diversity within breeding programs determined by SSR and the corresponding values calculated by AFLP ($r = 0.92$, $P = 0.004$). Moreover, a negative correlation was observed between kinship coefficients within breeding programs and genetic diversity values estimated by SSR ($r = -0.75$) and AFLP ($r = -0.79$, Table 3). These significant correlations indicate that these three independent sets of data likely reflect the same pattern of genetic diversity and validate the use of these data to analyze the partitioning of genetic diversity among Argentine wheat breeding programs.

Variation of Genetic Diversity in Time

Genetic diversity values estimated with SSR showed no significant differences among groups of cultivars released during the four different periods considered here (Table 4). Genetic diversity values for the different periods were very similar to the total variation on the basis of all the 105 cultivars (SSR, Table 4).

Genetic diversity estimates based on AFLP data also showed no differences between modern cultivars and those released in previous decades. However, significant differences ($P = 0.01$) were found between wheat cultivars released in the 1970s (PIC = 0.28) and those released in the 1980s (PIC = 0.34, Table 4).

DISCUSSION

Identification

The cultivars included in this study represent an almost complete spectrum of the bread wheat cultivars released in Argentina during the last 60 yr. The selected subset of 10 SSR discriminated among all 105 cultivars, as expected from the high average diversity (0.72) of the selected SSR (Brown et al., 1996) and the low level of inter-locus correlation.

The identification matrix based on these SSR provides a rapid and reliable method for cultivar identification that might be used for quality control in certified seed production programs, to identify sources of seed

Table 3. Comparison of average PIC values (±SE of the mean) and kinship coefficients (f) among sets of wheat cultivars released by different breeding programs. Means followed by different letters were significantly different (Tukey's $P < 0.05$).

Breeding program (Identification No. in Fig. 2)	Average PIC values				Average kinship	
	N†	SSR	N	AFLP	N	f
Inta (*1*)	37	0.72 ± 0.05 A	30‡	0.30 ± 0.02 A	28‡	0.11
Buck (*2*)	24	0.71 ± 0.05 A	24	0.30 ± 0.02 A	18	0.08
ACA (*3*)	7	0.66 ± 0.04 AB	8	0.24 ± 0.02 ABC	5	0.10
Klein (*4*)	19	0.61 ± 0.04 ABC	18	0.26 ± 0.02 AB	18	0.10
Thomas (*5*)	3	0.53 ± 0.04 ABC	3	0.16 ± 0.03 C	3	0.34
Nothrup King (*6*)	6	0.50 ± 0.08 BC	3	0.17 ± 0.03 C	5	0.17
Dekalb (*7*)	4	0.44 ± 0.08 C	4	0.18 ± 0.03 BC	4	0.26
Average value from all cultivars		0.72		0.30		0.12

† N number of cultivars included in each category.
‡ Different N was due to lack of data for some cultivars.

Table 4. Comparison of average PIC values (±SE of the mean) and kinship coefficients (*f*) among sets of wheat cultivars released in different decades. Means followed by different letters were significantly different (Tukey's $P < 0.05$).

		Average PIC values			Average kinship	
Period	*N*†	SSR	*N*	AFLP	*N*	*f*
<1969	14	0.70 ± 0.03 A	14	0.31 ± 0.02 AB	13‡	0.09
1970–1979	27	0.69 ± 0.04 A	23	0.28 ± 0.02 B	21	0.15
1980–1989	48	0.70 ± 0.05 AB	44	0.34 ± 0.02 A	37	0.08
1990–1995	16	0.68 ± 0.03 ABC	15	0.31 ± 0.02 B	11	0.02
Average value from all cultivars		0.72		0.30		0.09

† *N* number of cultivars included in each category.
‡ Different *N* was due to lack of data for some cultivars.

contamination, and to maintain pure and clean germplasm collections.

The average PIC value of these SSR is higher than the PIC value of 0.46 obtained with RFLP markers for the high molecular weight glutenins for the same set of cultivars. The high PIC of this set of SSR is partially related to the selection of highly polymorphic SSRs. However, the average PIC value of unselected SSRs also was 50% higher than the previous RFLP PIC estimate. This result is similar to that reported by Röder et al. (1995) and confirms the advantage of SSR compared with RFLP markers for genotype identification in wheat. As expected for dominant markers, AFLP markers showed lower PIC values than SSR. However, for species with no available SSRs, the AFLP technique provides a useful alternative for genotype identification compared to RFLP.

Genetic Relationships

Although these data are not extensive enough for a thorough characterization of the genetic relationships among this large set of cultivars, they can be used as a first draft of these relationships. The lack of clustering of cultivars by breeding program in Fig. 2 was expected on the basis of the available pedigree information. This information showed that breeding programs frequently use cultivars from other Argentine breeding programs as parental lines in their own crosses and that they use similar CIMMYT materials.

Correlations between the SSR and AFLP similarity matrices and the kinship coefficient matrix were low as expected from the low number of loci included in this study. However, the fact that this correlation was significant indicates that the information present in this small subset of molecular markers partially reflects the genealogical history of these cultivars. Consensus between dendrograms might be used to identify the conserved groups present in the different dendrograms. Low correlations between kinship coefficient and similarity matrices based on molecular markers also were reported in other studies including wheat, barley and oat (*Avena sativa* L.) cultivars (Graner et al., 1994; Plaschke et al., 1995; Schut et al., 1997; Bohn et al., 1999).

Variation of Genetic Diversity among Breeding Programs

Differences in genetic diversity detected between breeding programs that differ greatly in the number of released cultivars is expected. However, two important conclusions may be draw from this analysis. First, there are no significant differences in genetic diversity between the large public (INTA) and private breeding programs (Buck and Klein) in Argentina. Second, the average diversity within each of these three large breeding programs is very similar to the total genetic diversity present in the complete Argentine germplasm (Table 3). This suggests that each of the large breeding programs contains a representative sample of the complete diversity of the Argentine germplasm. This similar distribution of genetic variation among breeding programs is consistent with the limited clustering of cultivars by breeding program observed in Fig. 2.

Variation of Genetic Diversity during the Last Half Century

There is a general belief that modern breeding practices led to significant decrease of genetic diversity in modern cultivars (Vellvé 1993). There is concern that this erosion of the genetic variability might result in the reduction of the plasticity of the crops to respond to changes in climate, pathogen populations, agricultural practices, or quality requirements. However, the homogeneous genetic diversity values found in the Argentine bread-wheat cultivars released during the last half-century contradict this general belief. The variability available to the growers today is actually higher than the new variability released in the 1990s (Table 4). If cultivars released in the 1980s that are still grown in Argentina are included in the calculations, the diversity values for the 1990s increase from 0.681 to 0.722 for SSR and from 0.307 to 0.378 for AFLP.

Similar results to those reported here for the Argentine spring-wheat germplasm were recently reported for the dominant winter-wheat UK cultivars released between 1934 and 1994 (Domini et al., 2000). Analysis of SSR and AFLP markers for 55 UK wheat cultivars indicated that plant breeding in the UK has resulted in a qualitative, rather than a quantitative shift in the diversity over time. Souza et al. (1994) found similar results examining genetic diversity in spring wheat cultivars grown in the Yaqui Valley of Mexico and the Punjab of Pakistan. These regions also were beneficiaries of the semidwarf cultivars released during the Green Revolution in the early 1960s and no significant decrease in genetic diversity was observed.

A detailed analysis of the pedigree of modern Argen-

tine germplasm showed that variability was maintained by the use of derivatives of old Argentine cultivars and the permanent introgression of new materials from programs from other countries, particularly the material from CIMMYT. Alleles from old cultivars such as 38MA, Buck Atlantico, Buck Quequén, Ciano, Frontana, General Roca MAG, Lin Calel MA, Mentana, Nianari 59, Sinvalocho, and Sonora 64 are still present in modern Argentine breeding programs. The high genetic diversity values found in the public wheat-breeding program INTA and the private program Buck suggests that these two programs have made a major contribution to maintain the genetic diversity of Argentine wheat germplasm during the last half-century.

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Genetic Analysis and QTL Mapping of Cell Wall Digestibility and Lignification in Silage Maize

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ABSTRACT

Improving digestibility is a major goal for forage maize (*Zea mays* L.) breeding programs. Quantitative trait loci (QTL) affecting forage maize digestibility-related and agronomic traits were mapped and characterized in a set of recombinant inbred lines (RIL). Eleven traits were analyzed on whole plant samples: neutral detergent fiber (NDF), starch content (STC), crude protein content (CPC), acid detergent lignin (ADL), in vitro dry matter digestibility (IVDMD), in vitro cell wall digestibility (IVNDFD), in vitro digestibility of non-starch and non-soluble carbohydrate (IVDNSC), dry matter content (DMC), dry matter yield (DMY), mid-silk date (SILK), and plant height (PHT). Evaluation was performed among the RIL populations studied per se (RILps) and in combination with a tester (TC). The genetic variances (σ^2_g) were highly significant and, in most cases, greater than genotype × year interaction variances ($\sigma^2_{g\times y}$). Heritabilities ranged from 0.49 to 0.70 in RILps and from 0.12 to 0.58 in TC. Twenty-eight QTL were identified among TC by CIM, which explained individually between 3.3 and 20.2% of the phenotypic variation (R^2_p) for traits related to digestibility or agronomic performance. Twenty QTL were identified among RILps, which explained individually between 6.5 and 15.3% of the phenotypic variation (R^2_p). Seven of these QTL were common to TC and RILps. Cell wall digestibility estimates (IVNDFD or IVDNSC) were the traits with the highest number of QTL. In contrast, we detected only one QTL for dry matter digestibility (IVDMD). Thus, it may be useful to separate IVDMD into its two component parts, cell wall digestibility, which could be estimated from line per se values, and starch content. Characteristics such as IVDNSC or IVNDFD, coupled with QTL information, would be powerful tools in the search for genes involved in maize lignification or cell wall biogenesis.

SILAGE MAIZE is a major forage in dairy cattle feeding because of its high-energy content and good ingestibility. More than 3 500 000 ha are cropped in the European Union for silage making. Forage maize breeding in Europe has been based for a long time on the concept that the best hybrids for grain production were also the most suitable for forage use. However, it is now understood that selection for forage maize has to take into account specific criteria for feeding value (Gallais et al, 1976; Vattikonda and Hunter, 1983). Genetic variation for in vivo or in vitro digestibility has been reported in numerous studies, and improvement in dry matter digestibility should result from an increase in cell wall digestibility (Deinum and Struik, 1985; Dolstra and Medema, 1990; Barrière et al., 1992; Wolf et al., 1993; Coors et al., 1994; Argillier et al., 1995a). Silage digestibility is presently considered a major objective in silage maize breeding programs, but negative associations between digestibility and other agronomic traits (lodging and yield) were highlighted in some studies (Dhillon et al., 1990; Geiger et al., 1992; Argillier et al., 1995b; Barrière and Argillier, 1998). However, the genetic basis of digestibility-related traits and their relationships with other agronomic traits remains poorly documented. To date, only two published reports (Lübberstedt et al., 1997a, 1998) have examined QTL affecting digestibility traits on a whole plant basis. There have been no attempts to map QTL affecting digestibility traits for vegetative components of the plant.

The goal of our study was to determine the genetic basis of traits relating to agronomic and feeding value in silage maize. Genetic variation for these traits was investigated by means of a population of recombinant

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Abbreviations: ADL, acid detergent lignin; CIM, composite interval mapping; cM, centimorgan; CPC, crude protein content; DM, dry matter; DMC, dry matter content; DMY, dry matter yield; IVDMD, in vitro dry matter digestibility; IVDNSC, in vitro digestibility of non-starch and non-soluble carbohydrate; IVNDFD, in vitro cell wall digestibility; NDF, neutral detergent fiber; NIRS, near infra-red reflectance spectroscopy; PHT, plant height; QTL, quantitative trait locus/loci; RFLP, restriction fragment length polymorphism; RILs, recombinant inbred lines; RILps, RIL per se; SC, soluble carbohydrate; SILK, date of mid silking; STC, starch content; TC, test cross.