Theor Appl Genet (1993) 87:295-304

THEORETICAL AND APPLIED GENETICS

Evaluation of five strategies for obtaining a core subset from a large genetic resource collection of durum wheat

P. L. Spagnoletti Zeuli[1], **C. O. Qualset**[2]

[1] Department of Biology, University of Basilicata, 85100 Potenza, Italy
[2] Department of Agronomy and Range Science, University of California, Davis, CA 95616, USA

Received: 12 December 1992 / Accepted: 29 March 1993

Abstract. The use of plant genetic resources contained in a large collection may be enhanced by specifying subsamples, called core samples. Five strategies for selecting a core sample from a collection of 3000 durum wheat accessions were applied and evaluated using four qualitative and eight quantitative spike characters. Each of the following strategies generated about 500 accessions for the core sample: random, random-systematic according to chronology of entry of the accessions into the collection, stratified by country-of-origin, stratified by log frequency by country-of-origin, and stratified by canonical variables. The first three strategies produced samples representative of the whole collection, but the remaining two produced the desired effect of increasing frequencies from less-represented countries-of-origin for several characters. The stratified canonical sample increased phenotypic variances. The quality of core samples is dependent upon good passport and evaluation data to partition the collection. The multivariate approach is extremely useful, but requires considerable data from the whole collection. Ecogeographic origin may be used in the absence of evaluation data on several characters to select useful core samples.

Key words: *Triticum turgidum* L. durum group – Genetic resource conservation – Core collection

Introduction

Paradoxically, the major obstacle to full exploitation of many genetic resource collections is their large size. The incredibly large numbers of accessions that have been accumulated in gene banks to prevent erosion of genetic diversity are often poorly described. Their use for breeding purposes could be greatly increased if more information was available on the amount and kind of variation in these collections, but in most cases the resources needed to characterize thousands of entries phenotypically and genotypically are simply unavailable.

This task could be more easily fulfilled by the use of sub-sets of the whole collection, called active working collections by Harlan (1972) and core collections by Frankel and Brown (1984). A core subset should include a maximum of the genetic variation contained in the whole collection with a minimum of repetitiveness. Based on the neutral allele theory of Kimura and Crow (1964), Brown (1989a) simulated different scenarios for different numbers and frequencies of alleles at each locus variously distributed within a collection. On the basis of this analysis, Brown (1989a) proposed that a core subcollection should contain about 10% of the whole collection when the collection probably does not contain the total genetic variability for the species and a maximum of 3000 accessions for a "complete" collection. This sampling procedure should result in about an 0.85 probability of including 80% of the alleles that occur in the whole collection. Still, the unresolved issue is how to obtain a core subsample. Sampling efficiency can be improved whenever documentation is available on each accession. The geographical origin of accessions provides indirect evidence of diversity since adaptation to different environmental conditions can be

---

Communicated by A. R. Hallauer
Correspondence to: C. O. Qualset

inferred and accessions from the same origin can be assumed to share a larger portion of their gene pool than entries from different origins (Peeters and Martinelli 1989). Evaluation and characterization data based on strongly inherited characters of importance to the breeder can further improve the identification of phenotypic clusters within broad geographic groups. Stratification based on the identified clusters may improve the efficiency over simple random sampling. To avoid giving undue weight to large groups that are more likely to present a higher level of genetic redundancy, Growth and Roelfs (1987) and Brown (1989b) suggested that the number of accessions to be drawn should be proportional to the logarithm of the frequency in each group. Thus, there are several sampling strategies that can be applied, but how these may perform to meet the goals of a core subcollection depends on the species, the composition of the collection, and the type of characters of interest.

In this study we have compared the amount and the kind of variation for quantitative and qualitative characters of the wheat spike in samples drawn according to five strategies from a world collection of durum wheat (*Triticum turgidum* L. durum group). This collection included about 3000 accessions and is well-known for patterns of variation in spike traits according to geographic and character associations (Qualset and Puri 1974; Jain et al. 1975; Spagnoletti Zeuli and Qualset 1987, 1990).

Materials and methods

Basic data set

A large section (about 3000 entries) of durum wheat from the U.S. National Small Grains Germplasm Collection was planted in an irrigated field at Tulelake, California (41° 58′ N latitude, 121° 28′ W longitude; 1240 m elevation) in April 1970. The crop was mature about 10 September. A single row, with rows spaced 60 cm apart, was grown for each accession. Sentry and Oviachic 65 durum wheat cultivars, tall and short-statured, respectively, were grown periodically (more than 80 replicates) throughout the planting for estimation of heterogeneity in the field. The irrigated field proved to be very uniform with little environmental variance (Qualset et al. 1976 and unpublished). In addition, 86 accessions were planted at Tulelake in April 1986 from an independent seed source to verify the authenticity of the basic 1970 data set and the same group was planted in November 1986 in southern Italy at Gaudiano di Lavello (41° 061′ N latitude, 15° 52′ E longitude, 150 m elevation). In the latter two plantings, conditions appropriate for wheat culture were provided. Single-row plots with three replicates were grown.

Eighty-five accessions failed to head because of strong vernalization requirement and were not considered in this survey (Qualset and Puri 1974). As described by Spagnoletti Zeuli and Qualset (1987), one spike of average size per entry was visually selected from among five that were harvested at random from each accession for determining the number of spikelets, spike length (mm) from the collar to the tip of the apical spikelet, and awn length (mm) of the central spikelet. Kernel number/spike and the kernel weight/spike were obtained as the mean of the threshed grain from five spikes per accession. Awn length to spike length ratio, rachis internode length and weight/kernel were calculated from the basic data. Jain et al. (1975) reported qualitative characterization of spikes for the following traits: pubescent or glabrous glumes; black, brown, or white (including yellow) glumes; black or nonblack awns; and fertile or sterile basal spike node. Kernel color was scored as red, white (amber), and purple (blue).

This collection included entries from 26 countries-of-origin representing most of the area where durum wheat is grown. The number of entries from each country was highly variable ranging from a high of 834 from Turkey to several countries with ten or less. Spain, Russia, Ethiopia, Portugal, Turkey, and Tunisia contributed about 75% to the total collection.

Size of the core subcollection

The whole collection in our case was 3038 accessions and it seemed reasonable that an evaluator or breeder could process about 500 accessions easily. Thus, we chose a 16% proportion to represent the core, slightly more than Brown's (1989a) suggestion of 10%.

Sampling strategies

(1) *Random.* Sampling without replacement was done by a random number generator (SAS 1987). This resulted in a sample of 498 accessions.

(2) *Random-systematic by chronology.* For this strategy the whole collection was listed and planted in the field in numerical sequence by plant introduction (PI) number. This represents the order in which the accessions were accepted into the U.S. National Small Grains Collection. The accessions generally appeared in groups by country-of-origin, but one country-of-origin may appear in several places in the collection. The rule applied was to measure all check variety plots and every fourth accession except for certain groups of a large number of similar-appearing accessions from a single origin. In these cases (Portugal, Spain, Russia, and Turkey) every fifth, seventh, or tenth accession was measured. An analysis of all measured traits from this subsample of 734 (approximately 24.1%) showed that this represented the means and ranges of the whole population (Spagnoletti Zeuli and Qualset 1990). This sampling strategy is thus both systematic and random because no predesignation of accessions was imposed and the accession sequence for a particular group represents one of the possible randomization sequences.

(3) *Random-stratified by geographic origin and frequency.* This strategy was applied by fixing the total number of selected accessions (16%) and then selecting at random a fixed number of accessions from each country-of-origin proportioned to the number of accessions from each country. A random number generator (SAS 1987) was used to assemble 498 accessions.

(4) *Random-stratified by log frequency of accessions by geographic origin.* This strategy is identical to strategy 3, but the logarithm of the frequency of accessions from each country was used to establish the number of accessions from each country. This approach ensures that countries providing few accessions are represented in the sample and also that those countries with extraordinarily large number of accessions contributed relatively fewer accessions to the core subsample. The SAS (1987) random number generator produced 495 accessions by this strategy.

(5) *Random-stratified by canonical variables.* The rationale for this strategy is based on the concept that preexisting information about the collection can be used to stratify the accessions on phenotypic as well as geographic bases. Previous analysis of eight characters in the whole collection (Spagnoletti Zeuli and

Qualset 1987) revealed five cluster groups after applying canonical discriminant analysis methods according to Seal (1964) and Pimentel (1979). These canonical variables accounted for at least 80% of the variation with a consistent reduction from the original model. The clusters corresponded to groupings according to centers of diversity (Spagnoletti Zeuli and Qualset 1987). This classification proved to be little affected by sample size or by the introduction flag leaf measurements to the data set (Spagnoletti Zeuli and Qualset 1990). The canonical variables were computed for 3038 accessions. The first three canonical variables were plotted for each accession. Cells of one unit in each of the three dimensions were chosen as units for selection of a sample of accessions. Entries that occurred in each cell were stratified by country-of-origin and about 10% were randomly selected. The proportion of accessions from each country in each cell was not the same for all the countries represented because there was deliberate bias toward including a higher proportion of accessions from countries having low frequencies of accessions. For example, if in one cell 60 accessions were from Turkey, ten from Italy, three from Ethiopia, and one from India, the number of accessions selected would be six, three, three and one, respectively. This procedure produced 498 accessions in the subsample.

Results

In Table 1 are shown interlocation or interyear correlations for six characters observed in the sample of 86 genotypes from different geographical origins over three environments. For all characters, the correlation coefficients were significant with most values larger than 0.38. Also the structure of covariation between characters within each environment was very similar (Table 2). Thus, stability of most of these characters over environments is relatively high and can, therefore, be used in the study of variation in large collections where replicated experiments are impractical.

Frequency of entries from each geographic origin

The number and percentages of accessions from each country-of-origin in the world collection are shown in

Table 1. Correlation coefficients between three environments (Tulelake 1977, 1986; Gaudiano 1987) for six quantitative characters of the spike observed on 86 durum wheat entries

Character	Tulelake 1977 with Tulelake 1986	Tulelake 1977 with Gaudiano 1987	Tulelake 1986 with Gaudiano 1987
Spike length	0.56**	0.48**	0.86**
Kernel number	0.25*	0.38**	0.66**
Kernel weight/spike	0.39**	0.47**	0.67**
Weight/kernel	0.71**	0.73**	0.84**
Total spikelet number	0.39**	0.28**	0.41**
Rachis internode length	0.68**	0.55**	0.85**

* $P \leq 0.05$, ** $P \leq 0.01$

Table 2. Correlation coefficients among six quantitative spike characters observed on 82 durum wheat genotypes within three environments (Tulelake 1977, 1986; Gaudiano 1987)

Character	Location	Kernel number/spike	Kernel weight/spike	Weight/kernel	Spikelet number	Rachis internode length
Spike length	Tulelake 1977	0.05	0.01	−0.06	0.19	0.84**
	Tulelake 1986	0.12	0.05	−0.04	0.34**	0.65**
	Tulelake 1987	0.11	0.13	0.13	0.14	0.81**
Kernel number per spike	Tulelake 1977		0.83**	−0.02	0.44**	−0.19
	Tulelake 1986		0.80**	0.15	0.26*	−0.13
	Gaudiano 1987		0.81**	0.17	0.73**	−0.31*
Kernel weight/spike	Tulelake 1977			0.53**	0.36**	−0.17
	Tulelake 1986			0.70**	0.13	−0.08
	Gaudiano 1987			0.70**	0.48**	−0.14
Weight/kernel	Tulelake 1977				0.05	−0.01
	Tulelake 1986				−0.15	0.08
	Gaudiano 1987				−0.05	0.18
Rachis internode length	Tulelake 1977					−0.36**
	Tulelake 1986					−0.48**
	Gaudiano 1987					−0.44**

* $P \leq 0.05$, ** $P \leq 0.01$

Table 3 along with the frequency deviations from the world collection frequencies observed in the five subsamples; χ^2 values calculated to compare frequencies in the whole collection to the samples are also reported. The deviations for country-of-origin were compared to the whole population for each sampling strategy by χ^2 analysis. The random and random-stratified by origin samples were not significantly different from the world collection; however, the other strategies produced frequency distributions according to origin that differed from the whole population. This was an expected and desired result.

In the systematic sample some origins had proportionally more (Bulgaria, Israel, Egypt, Ethiopia, USA) or less (Poland, Turkey, Spain) accessions in the sample than in the whole population. It should be noted that a significant reduction was not obtained for accessions from USSR and Portugal despite the fact that there were many accessions from these origins and that accessions in the samples appeared at proportionally lower frequencies. This is probably due to the fact that batches of accessions from these countries with consecutive PI numbers were smaller than for Turkey and Spain, thus reducing the impact of the selection of every 4th or 5th accession for the systematic sampling strategy.

In the stratified log sample the frequency deviations by country from the world collection were larger than for any other sampling strategy ($\chi^2 = 583$). The percentages of accessions selected from different countries ranged from 2.3% for Yugoslavia to 6.4% for Turkey. These origins were, respectively, the least (0.4%) and the most (27.5%) represented in the world collection. This strategy was the most successful one in producing the desired effect of lowering the representation from countries with high numbers of accessions, especially Spain, Turkey, and Russia.

In the stratified canonical sample, significant differences in frequencies from the world collection were evident for both the most- and least-represented coun-

Table 3. Proportion of entries from 26 countries of origin in a world collection of durum wheat and percentage deviations in five samples drawn with different strategies. The χ^2 values are for departure from proportions in the world collection

Country of origin	No.	World % collection	Deviation (%) from world collection frequency				
			Random	Systematic random	Stratified by origin	Stratified by log of origin	Stratified by canonical variables and origin
Poland	18	1.6	−1.4	−1.2	−1.0	1.2	−0.8
France	40	1.3	−0.1	0.5	0.1	2.2	0.7
Portugal	211	6.9	0.2	−0.2	−0.1	−1.8	1.4
Spain	459	15.1	1.3	−2.8	−0.2	−9.3	−2.6
Italy	67	2.2	0.8	0.5	0.0	1.8	2.2
Hungary	44	1.4	−0.4	0.8	0.2	2.2	0.8
Yugoslavia	11	0.4	0.0	0.0	0.0	1.9	0.4
Bulgaria	51	1.7	0.5	1.1	0.1	2.0	0.5
Greece	37	1.2	0.4	0.3	0.0	2.2	0.4
Ethiopia	268	8.8	−0.1	1.0	−0.2	−3.5	−0.5
Algeria	31	1.0	−0.4	0.4	0.0	2.3	−0.4
Morocco	27	0.9	−0.7	0.5	0.1	2.2	−0.5
Tunisia	145	4.8	−0.4	−0.7	0.0	−0.1	1.9
Egypt	34	1.1	0.1	1.1	0.1	2.3	−0.3
Cyprus	29	1.0	1.0	0.0	0.0	2.2	0.6
Lebanon	13	0.4	−0.2	0.3	0.2	2.0	0.0
Jordan	42	1.4	0.6	0.5	0.0	2.1	0.8
Israel	23	0.8	−0.2	0.8	0.0	2.2	0.2
Syria	32	1.1	0.5	0.4	0.1	2.2	0.5
Turkey	834	27.5	−0.4	−6.4	−0.6	−21.1	−11.1
Iraq	34	1.1	−0.3	−0.5	0.1	2.3	0.7
Iran	69	2.3	−0.5	0.6	0.1	1.7	0.1
Afghanistan	37	1.2	−0.4	0.6	0.0	2.2	1.6
Russia	361	11.9	−1.4	−0.9	−0.3	−6.3	−2.6
India	65	2.1	0.1	0.8	0.1	1.9	2.5
USA	56	1.8	0.2	1.1	0.0	−1.8	2.0
Number	3038		495	734	498	495	495
χ^2			21.23	57.15**	1.01	582.94**	93.76**

** $P < 0.01$

Table 4. Means, standard errors, variances, and coefficients of variation for quantitative characters of the spike in a world collection of durum wheat and for five sampling strategies. Means and variances were tested for significant differences from the world collection values

Strategy	Spike length				Rachis internode length				Awn length				Awn/spike length ratio			
	Mean	SE	Var	CV	Mean	SE	Var	CV	Mean	SE	Var	CV	Mean	SE	Var	CV
World collection	76.0	0.221	142.75	15.7	4.11	0.013	0.491	17.0	122.8	0.535	862.95	23.9	1.66	0.009	0.223	28.4
Random sample	75.6	0.516	125.69	14.7	4.08	0.030	0.435	16.2	122.1	1.343	888.86	24.4	1.66	0.021	0.211	27.6
Systematic	75.4	0.455	139.51	15.7	4.10	0.027	0.484	17.0	121.4	1.144	909.03	24.8	1.66	0.018	0.227	28.7
Startified by origin	75.9	0.543	141.14	15.7	4.10	0.031	0.470	16.7	122.3	1.331	878.13	24.2	1.66	0.022	0.220	28.2
Stratified by log of origin	75.1	0.558	147.44	16.2	4.14	0.033	0.522	17.5	125.0	1.292	816.34	22.9	1.73	0.022	0.26	27.5
Stratified by canon var/origin	76.0	0.602	176.60**	17.5	4.12	0.035	0.608**	19.0	119.5	1.507	1105.69**	27.8	1.63	0.024	0.290**	33.10

Strategy	Spikelet number				Kernel number				Kernel weight/spike				Weight/kernel			
	Mean	SE	Var	CV	Mean	SE	Var	CV	Mean	SE	Var	CV	Mean	SE	Var	CV
World collection	18.6	0.038	4.439	11.3	38.7	0.151	68.92	21.5	2.14	0.010	0.307	25.9	55.3	0.143	61.98	14.2
Random sample	18.6	0.092	4.196	11.0	38.6	0.360	64.05	20.8	2.14	0.024	0.291	25.3	55.2	0.351	60.89	14.1
Systematic	18.5	0.078	4.293	11.2	38.6	0.322	72.69	22.0	2.12	0.021	0.308	26.2	54.8	0.297	61.74	14.3
Stratified by origin	18.6	0.097	4.692	11.6	39.2	0.382	72.82	21.8	2.17	0.025	0.322	26.1	55.3	0.367	67.19	14.8
Stratified by log of origin	18.2**	0.090	4.011	11.0	38.8	0.395	73.80	22.2	2.15	0.024	0.290	25.1	55.5	0.370	67.84	14.8
Stratified by canon var/origin	18.6	0.099	4.760	11.7	38.7	0.352	61.51	20.3	2.10	0.025	0.308	26.4	54.3**	0.361	63.61	14.7

$*P < 0.05$, $**P < 0.01$

tries in the world collection (Table 3). For example, fewer accessions were included from Turkey and Spain and more accessions from Italy, India, Afghanistan, and USA. Accessions from Turkey were 16.4% of this sample and significantly less than the 27% observed in the whole collection. This dramatic reduction of the entries from this origin was balanced by a proportional increase of the number of entries for most other origins. The reduction of entries from Turkey was very much needed since phenotypic uniformity was observed in the field and redundancy was suspected. This strategy gave a much larger reduction in the number of accessions from Turkey than did the systematic sampling strategy.

Univariate statistics

Sample means for the various sampling strategies were significantly different from the whole population mean only for kernel weight and awn length in the canonical sample and for awn to spike length ratio and spikelet number per spike in the stratified log sample (Table 4). Sample variances were significantly smaller ($P < 0.05$) than in the world collection for spike and rachis inter-

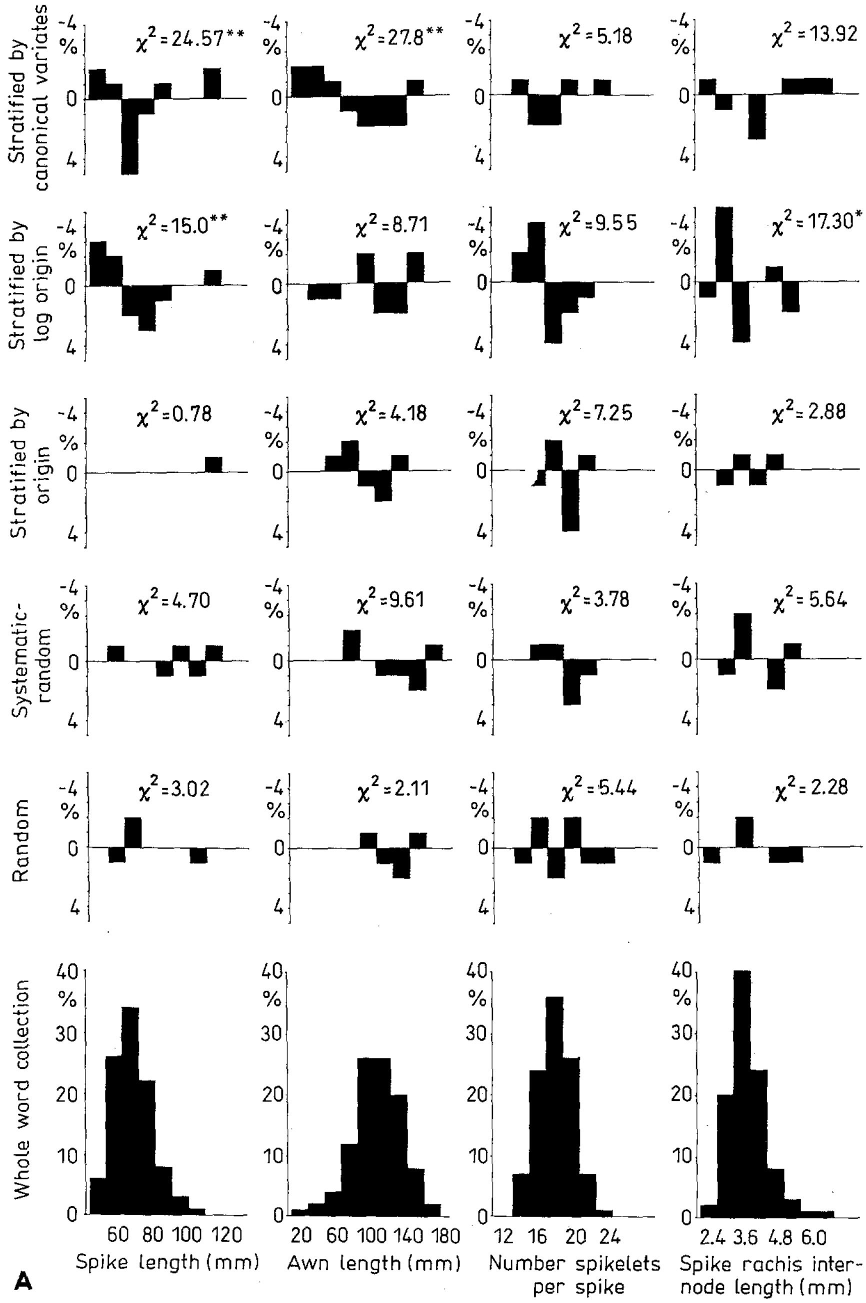

Fig. 1. (*Continued*)

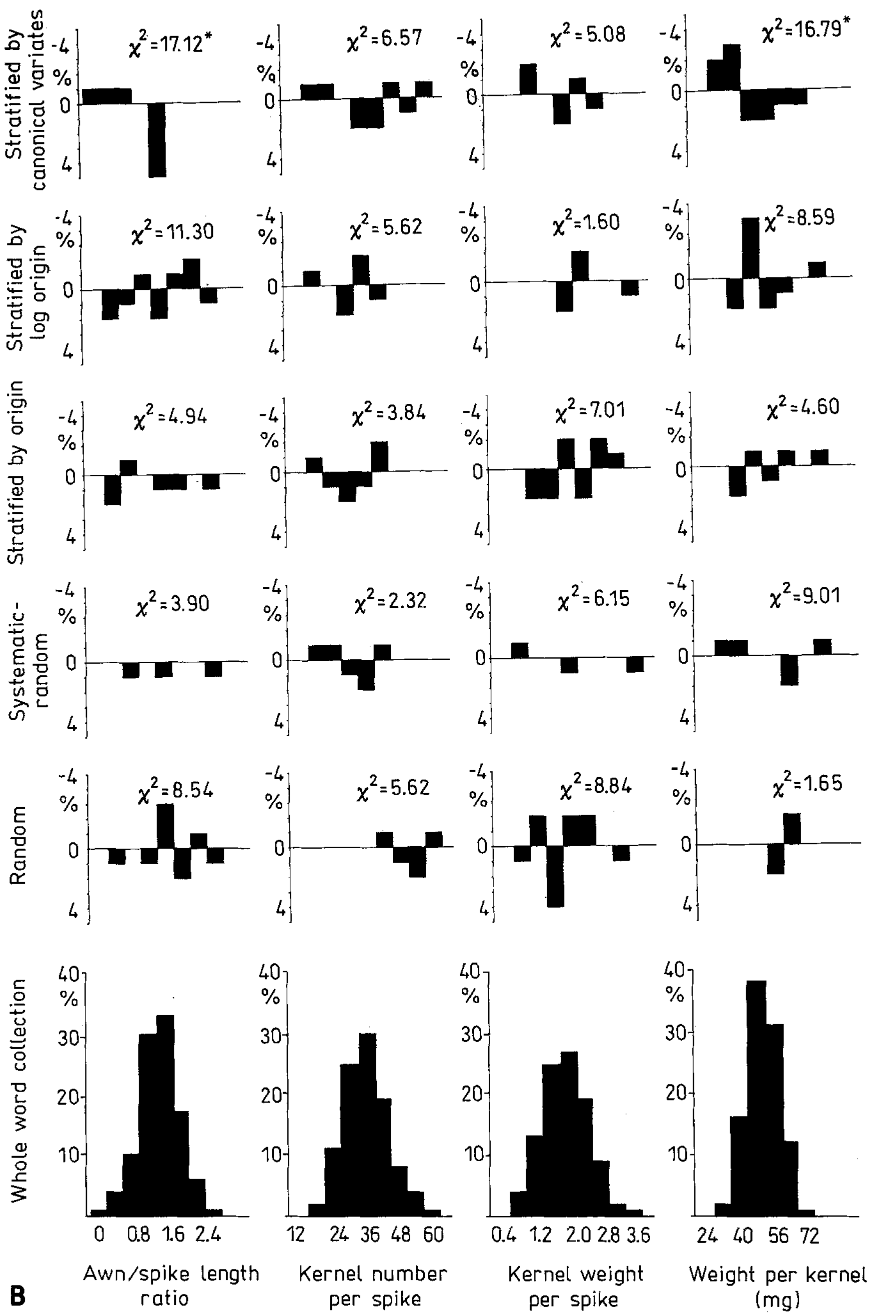

Fig. 1A, B. Frequency distributions for eight quantitative characters of the spike in a world collection of durum wheat and frequency changes in five samples drawn with different strategies; χ^2 values are for tests of departure of sample frequencies from world collection frequencies

node length in the random sample and for kernel number per spike for the canonical sample. Variances were significantly larger only for awn length and spike and rachis internode length in the stratified canonical sample. Awn length was highly correlated with the first canonical variable while the other spike characters showed a high correlation with the second canonical variable. These two variables accounted for 73% of the total variance in the whole collection (Spagnoletti Zeuli and Qualset 1987).

Frequency distributions for quantitative traits

The frequency distributions of quantitative spike traits were obtained for each sampling strategy and compared to the world collection (Fig. 1). The stratified canonical strategy produced significant deviant distributions for spike length, awn length, awn to spike length ratio, and kernel weight, while the stratified log strategy gave deviations for spike length and rachis internode length. No significant effects on the frequency distributions were found for any character for the other three sampling strategies.

The stratified canonical strategy increased the frequencies of the least-represented classes while the stratified log sample resulted in a reduction in one little-represented spike internode length class and an increase in one already well-represented class. These characters showed a high correlation with the first canonical variable which accounted for 46% of the total variance. χ^2 values, although not significant, were higher in the stratified canonical sample for five out of eight characters, thus supporting the idea that this strategy increases the frequency of less-represented classes for most characters.

The frequency distributions within the specific geographic origins that showed a significant increase or decrease of the number of entries included in the canonical sample, such as Turkey, Italy, India, Afghanistan, and USA, were compared to the whole collection and to the distributions in the four other derived samples. Entries from these countries represented a little more than 30% of all entries in the world collection and in all other samples, except the stratified log sample, where they were 22%. In this subset of the samples the frequencies were significantly different only for the stratified canonical sample for all quantitative characters observed, with the exception of kernel weight per spike and for the stratified log sample for awn length, kernel number per spike, and kernel weight. This means that redundancy associated with the country-of-origin could be reduced by the stratified canonical strategy and only occasionally reduced with the stratified log strategy.

Frequency distribution of qualitative traits

Phenotypic frequencies for four qualitative characters observed on the spike (Table 5) were compared for each sampling strategy with the world collection frequencies. Since these characters had not been used to define any of the tested sampling strategies, they give a measure of the effects of the sampling strategies on other loci.

These traits appeared in the whole collection at relatively high frequencies (Table 5), with only brown glume color and purple kernel color at 5% or lower frequencies. Thus, the high-frequency traits would be expected to appear in all sampling strategies while those two low-frequency traits may be vulnerable to loss in the 500-accession samples, depending, of course, on their geographic distribution. Only awn color showed significantly-altered frequencies of types among the five traits and five sampling strategies. Although not significant, the χ^2 values for the stratified

Table 5. Frequencies (%) in world collection, deviations in frequencies for qualitative characters and χ^2 value for comparison of sample to world collection frequencies

Character	World collection frequency	Random	Systematic random	Stratified by origin	Stratified by log origin	Stratified by canonical var. and origin
Glume pubesence						
Present	80	+1	−1	–	−3	−1
Absent	20	−1	+1	–	+3	+1
χ^2		0.11	1.54	0.02	3.74	0.88
Glume color						
White and yellow	83	−1	−1	+2	−3	−2
Brown	5	–	–	−1	+2	+1
Black and gray	12	+1	+1	−1	+1	+1
χ^2		0.11	0.64	1.51	2.61	1.17
Awn color						
Black	58	+2	–	–	−6	−4
Not black	42	−2	–	–	+6	+4
χ^2		0.40	0.08	0.01	7.31**	2.86
Basal spike node						
Fertile	39	+1	–	+4	+1	+2
Not fertile	61	−1	–	−4	−1	+2
χ^2		0.39	0.06	2.46	0.21	0.44
Kernel color						
White or amber	76	–	−1	+2	−1	−3
Red or brown	22	–	+1	−2	+1	+3
Purple or blue	2	+1	–	–	–	–
χ^2		1.43	1.94	0.76	0.24	2.07

** $P \leq 0.01$

log and stratified canonical sampling strategies were generally larger than for the other three samples.

Discussion

Central to the problem of how to draw samples from large genetic resource collections is the purpose for which the collection is to be sampled. The core sample concepts of Harlan, Frankel, and Brown were primarily proposed to meet the needs of breeders and others who wish to identify unique gene resources. For this purpose a random sample is not desired. Rather, the sample should be biased to assure the inclusion of accessions from poorly represented geographic areas or based on other crop-specific considerations. However, if the purpose is to study the amount and structure of phenotypic and genotypic variability in a crop species, then it is important that the core sample be representative of the collection as a whole. This, of course, does not address the issue of whether the collection itself was constructed adequately to address such issues. The present study was motivated by utilitarian goals whereby a genetic resource collection should be sampled and be available to search for specific traits and/or the genetic diversity underlying traits of importance for plant breeding or other uses.

As proposed by Frankel and Brown (1984), the sampling strategy to obtain a core sample should maximize the diversity in the sample while attempting to reduce the redundancy of identical genotypes. Practically, this means that frequencies of entries in the most represented classes should be reduced while increasing the frequencies of the most rare types. Thus, plant breeders might then use this core sample (1) as a source of exotic germplasm to widen the genetic base of their breeding population in the hope of identifying new yield-promoting genes, or (2) to discover a specific characteristic and in which portion (e.g., geographical origin) of the whole collection it is likely to be found. In both cases the core sample must include the most diverse genotypes.

Thus, the sampling strategy to be adopted should provide a mechanism to ensure that the least-represented types are included. Sampling theory is the basis of any strategy, but information on the amount and distribution of variation within a collection should be used whenever available (Brown 1989a).

Our study tests the performance of different sampling strategies using evaluation data, including both quantitative and qualitative characters, from a real genetic resources collection. Despite the fact that our results may be specifically appropriate for a durum wheat collection, some general conclusions are drawn that may be instructive in approaching this question with other crop plants. The five strategies simulated different situations that may be presented to a user of a large collection: (1) only an accession number is available, (2) only the country of origin is available, (3) some phenotypic judgment on the degree of similarity can be made by observing the plants in the field, or (4) detailed phenotypic evaluation data are available for a few quantitative characters.

The kind and the amount of variation included in the samples obtained using the first three strategies (random, stratified random, and systematic random) applied in this study gave a good approximation to the composition of the world collection. Stratification of the sample using the log-of-origin frequency (Brown 1989b), as expected, dramatically changed the frequency of entries from each country while no changes in the phenotypic variances were observed for eight quantitative characters. The effect on the relative frequencies of phenotypic classes appeared sporadic: for some characters the number of entries was increased in the least-frequent classes and reduced in the most frequent, in some others the opposite occurred. Therefore, this strategy had the desired effect of ensuring representation of little-represented countries of origin in the sample, but its effect on specific traits was not predictable. The latter may be expected because the frequency distributions for the traits themselves were not a criterion for selection.

As expected, the frequencies of the rare classes were increased more consistently with the stratified canonical strategy which, however, can only be used if data are available for some representative traits. This strategy was effective in increasing the phenotypic variances in the sample for most characters. The larger variances were due mostly to the increase of the less-frequent accessions and a decrease of the most-frequent ones, thus flattening the frequency distribution. Despite the fact that changes in the frequencies of the four qualitative characters were not significant, Table 5 shows that the frequencies of the rarest classes were increased in six of seven classes, and no change was found for the rarest morph examined in this study. Quantitative characters are likely to be controlled by genes distributed throughout the whole genome and changes of the gene frequencies at some of those loci should result in changes at loci linked to them.

If the canonical-based stratification approach is to be useful, consideration must be given to which characters should be included in the multivariate analysis. Many of the characters identified as descriptors by IBPGR are potentially usable, some of these are single-gene characters and others are multigenic. Spike characters and flag leaf dimensions of wheat are easily measured and relatively less affected by environmental factors than many other characters. In addition, flowering time and plant height may be useful, but the nature of the environmental conditions must be specified and considered in the interpretation of the results. The spike characters permit evaluation of kernel number and weight, two grain yield components which are also important indicators of evolutionary history (Spagnoletti Zeuli and Qualset 1990). Spike characters are often used as on-site indicators of diversity during collection expeditions. We therefore recommend that these characters be included in the routine characterization of accessions because they can be adequately measured in one-replicate regeneration plantings.

We agree that sub-samples of large collections must be taken when a search for specific traits is begun. The core sample concept provides a framework for drawing the sample. The core sample may be regarded as a permanently available sample or it may be one which is created in response to a specific need. Such "floating core samples" would be made by using prior knowledge about the whole collection and the sample would be specified by a certain set of descriptors. In other cases, the likely agroecological features that would favor evaluation of the trait can be specified and a sample of accessions drawn from sections of the collection which represent those features. In practice, both permanent and floating core samples are appropriate, depending on the sophistication of the genetic resources collection in terms of the amount of data available and the availability of a working database management system. The log-origin frequency and canonical stratification strategies applied here have useful features for designing a core sample. However, these and other strategies for defining a core sample of accessions should, in Harlan's (1972) words with reference to sorghum, be "carefully chosen and stratified by rare, subrare, geographical distribution, and ecological adaptation". That is, a wide range of information about the collection is needed, but knowledge of geographic origin remains as a highly critical source of information in genetic resources management.

Development of effective core samples will be difficult without access to evaluation and characterization data. Unfortunately, little or no data are available in many collections. We suggest, though, that in these cases a core collection should be extracted. To accumulate enough evaluation data when resources are limited could be simply unfeasible. In these cases where even geographic origin data are missing a core sample can be produced using a simple random sampling strategy and the first stage of evaluation could concentrate on this core sample. This will have several advantages: (1) evaluation programs could be matched in scope to the resources available, (2) accessions of immediate interest for breeding purposes can be identified, (3) the structure of covariation among characters can be studied and the more informative characters can be identified, (4) characters can be chosen that allow a better discrimination among groups of accessions and describe the distribution of variation in the collection, (5) the practical experience accumulated at this stage will help in defining an efficient strategy for the evaluation of the whole collection, and (6) this partial description will very likely raise the breeders interest in the collection and will help in obtaining the resources needed for more extensive evaluation programs.

Acknowledgements. We acknowledge financial support for this work through a grant from the International Board for Plant Genetic Resources and a travel grant to P. L. Spagnoletti-Zeuli from the Italian National Research Council. The U.S. National Small Grains Collection staff at Aberdeen, Idaho, have provided assistance throughout this work.

References

Brown AHD (1989a) Core collections: a practical approach to genetic resources management. Genome 31:818–824

Brown AHD (1989b) The case for core collections. In: Brown AHD, Frankel OH, Marshall DR, Williams JT (eds) The use of plant genetic resources. Cambridge University Press, Cambridge, pp 136–156

Frankel OH, Brown AHD (1984) Plant genetic resources today: a critical appraisal. In: Holden JHW, Williams JT (eds) Crop genetic resources: conservation and evaluation. Allen and Unwin, Winchester, Massachusetts, pp 249–268

Growth JV, Roelfs AD (1987) The concept and measurement of phenotypic diversity in *Puccinia graminis* on wheat. Phytopathology 77:1395–1399

Harlan JR (1972) Genetic resources in sorghum. In: Rao NGP, House LR (eds) Sorghum in the seventies. Oxford and IBH, New Delhi, pp 1–13

Jain SK, Qualset CO, Bhatt GM, Wu KK (1975) Geographical patterns of phenotypic diversity in a world collection of durum wheats. Crop Sci 15:700–704

Kimura M, Crow JF (1964) The number of alleles that can be maintained in a finite population. Genetics 49:725–738

Peeters JP, Martinelli JA (1989) Hierarchial cluster analysis as a tool to manage variation in germplasm collections. Theor Appl Genet 78:42–48

Pimentel RA (1979) Morphometrics. The multivariate analysis of biological data. Kendall/Hunt, Dubuque, Iowa

Qualset CO, Puri YP (1974) Sources of earliness and winter habit in durum wheat. Wheat Inf Serv 38:13–15

Qualset CO, Carvalho FIF, Vogt HE (1976) A comparison of the theoretical variance in tetraploid wheat leaf traits with implications on optimum sampling size. Agron Sulriograndense 12:97–109

SAS Institute Inc. (1987) SAS Application Guide, 1987 edition. SAS Institute Inc., Cary, North Carolina

Seal H (1964) Multivariate statistical analysis for biologists. Methuen, London

Spagnoletti Zeuli PL, Qualset CO (1987) Geographical diversity for quantitative spike characters in a world collection of durum wheat. Crop Sci 27: 235–241

Spagnoletti Zeuli PL, Qualset CO (1990) Variation for flag leaf dimensions in durum wheat germplasm from different geographic origins. Plant Breed 105:189–202