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Practical considerations for maintaining germplasm in maize

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Abstract The main goals of genetic resource management are to acquire, maintain, distribute, characterize, regenerate, preserve, evaluate, and utilize the genetic diversity of crops and their wild relatives. The objectives of this study for *ex-situ* conservation of maize (*Zea mays* L.) are to review and describe: (1) practical regeneration methods that are based on population genetic theory; (2) practical problems encountered in choosing core subsets of a maize collection. Whenever possible, regeneration procedures should control the number of pollen parents (male gametes; through controlled hand pollination) and the number of female parent gametes (by harvesting equal numbers of kernels from each seed plant). When the number of pollen and seed parents are controlled during regeneration, the effective population size (N_e) is twice the size of the original population (N). Examples of practical methods for controlling the number of male and female parents are presented. The procedure involves random-paired plant crosses and taking equal numbers of seeds from each maize ear. To form a core subset, accessions of a maize race are subdivided through a stratified sampling procedure. Delineation of a core subset from a Tuxpeño racial collection is described as an example.

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Introduction

The principal goals of *ex situ* genetic resource management are to acquire, maintain, distribute, preserve, characterize, evaluate, and enhance genetic diversity. Germplasm management programs should pursue all these goals rather than simply acquiring and storing accessions (Goodman 1990; Shands 1990).

Genetic resource managers face many challenges when regenerating, characterizing, and evaluating germplasm. These challenges include maintaining the accession's original genetic structure by avoiding, as much as possible, contamination via outcrossing or mixtures of seeds/propagules, and any loss of genetic diversity due to populational bottlenecks and subsequent inbreeding. To preserve each accession's genetic diversity efficiently, an optimal sample size must be used for regeneration, as pointed out by Crossa (1989) and many others. When sample sizes are very large, regeneration is expensive, whereas small sample sizes may result in the loss of relatively rare alleles.

Core subsets are formed to maximize the efficiency of germplasm evaluation as well as the genetic diversity in a collection. Frankel and Brown (1984) and Brown (1989a, b) describe how to assemble a core subset using the accessions' morphological and agronomic characteristics. When forming a core subset, it is essential (1) to know the optimal number of accessions for preserving most of the alleles in the collection; and (2) to use appropriate criteria for choosing accessions for the core subsets.

The objectives of this paper are to review and describe: (1) practical regeneration methods, based on population genetic theory, for regenerating populations and (2) practical aspects in choosing core subsets of a bank collection. As in an earlier study (Crossa 1989), these investigations focus on outbreeding species like maize.

Practical methods for regenerating maize accessions according to population genetic principles

Population genetic principles

The fitness of individuals in a natural outbreeding population of size N varies, as do the various numbers of gametes (offsprings) an individual contributes to the subsequent generation. Accordingly, in actual populations the effective size (N_e) usually differs from the actual number of adults of reproductive age (N).

At least five factors contribute to this difference: (1) unequal numbers of individuals (or families) per generation, (2) unequal numbers of individuals of each sex, (3) unequal numbers of gametes produced per individual (or family) that contribute to the next generation, (4) non-random mating which reduces N_e because of the additional genetic drift due to gene frequency correlation in the parents (Caballero and Hill 1992), and (5) selection which also reduces N_e (Robertson 1961).

In an idealized population of infinite size, it is assumed that gametes unite at random to form the next generation, such that all individuals (or families) contribute the same number of surviving progeny. In reality, the number of progeny contributed by individual families varies and greatly reduces N_e. To ameliorate this problem, germplasm collection and regeneration procedures should attempt to equalize the number of progeny contributed per individual (or family) to the next generation.

In a non-ideal population of size N and having gametes drawn at random from the parental population, the number of gametes contributed by the parents (k) often follows a Poisson distribution, and $s^2(k)=\bar{k}$ (Crossa and Vencovsky 1994). Consider that, with (1) k_m and k_f representing the male and female gametes contributed per individual, respectively, (2) a mean number of gametes $\bar{k}=\bar{k}_m+\bar{k}_f$ (where $\bar{k}_m=\Sigma k_m/N_m$ and $\bar{k}_f=\Sigma k_f/N_f$, and where N_m and N_f are the total number of male and female individuals, respectively) such that $\bar{k}_f=(1/2)\bar{k}$ and $\bar{k}_m=(1/2)\bar{k}$, and (3) no covariance between contributed female and male gametes, the variance in the number of gametes is given by $s^2(k)=s^2(k_f)+s^2(k_m)=\bar{k}_f+\bar{k}_m=(1/2)\ \bar{k}+(1/2)\bar{k}=\bar{k}$ (Crossa and Vencovsky 1994) where $s^2(k_f)$ and $s^2(k_m)$ are the variance of the number of female and male gametes, respectively. However, the value of $s^2(k)$ may be more than $s^2(k_f)+s^2(k_m)$ when individuals are lost from the population before reproducing (e.g., seeds do not germinate, Crossa and Vencovsky 1994).

Crow and Kimura (1970) developed two concepts of N_e. One is the inbreeding effective population number [$N\hat{e}_{(i)}$] defined by the number of individuals (N) in the parental generation and related to the change in probability of identity by descent. Effective population size can also be defined as a function of variance of population size [$Ne_{(v)}$] and is related to the number of gametes (offspring) contributed by particular individuals in a population. If the present generation is t, $Ne_{(i)}$ is calculated for previous generation t-1. In contrats, $Ne_{(v)}$ is the expected N_e of the population in the next generation t+1; accordingly, in germplasm regeneration, $Ne_{(v)}$ is more important than $Ne_{(i)}$.

$Ne_{(v)}$ is defined as

$$Ne_{(v)}=2N_t/[\{s^2(k)/\bar{k}\}(1+\alpha_{t-1})+(1-\alpha_{t-1})] \quad \text{(Eq. 1)}$$

where N_t is N in generation t, and α_{t-1} is a measure of departure from Hardy-Weinberg proportions. Equation 1 has been corrected by Caballero and Hill (1992) for cases where inbreeding is due to mating of close relatives without selfing.

In a random mating population in Hardy-Weinberg equilibrium, $\alpha_{t-1}=0$ and the variance effective population size is

$$Ne_{(v)}=2N_t/[1+s^2(k)/\bar{k}] \text{ or} \quad \text{(Eq. 2)}$$

$$Ne_{(v)}=2N_t/[1+\{s^2(k_f)+s^2(k_m)\}/\bar{k}] \quad \text{(Eq. 3)}$$

When numbers of male and female parents are controlled, then $s^2(k)=0$ [$s^2(k_f)=0$ and $s^2(k_m)=0$] and Eq. 3 becomes $Ne_{(v)}=2N_t$, or twice the size of the census population. Although equalizing k_m and k_f is unlikely in nature, this equality can be secured when regenerating germplasm accessions (Gale and Lawrence 1984; Crossa 1989).

In general, N_e depends on (1) the crossing system and (2) how male and female gametes (parents) are sampled (Hallauer and Miranda 1981). Crossa and Vencovsky (1994) presented appropriate equations for calculating variance effective population size as related to germplasm regeneration for different male and female parent control strategies.

When the accession to be regenerated is monoecious, each plant can serve as both a male and female parent. Three cases are considered.

Case 1. If pollination is not random, equal numbers of seeds are secured from each parent, and k_m is controlled by making plant-to-plant crosses (with or without reciprocals) or chain crosses, then equal numbers of male and female plants are represented, $s^2(k_f)=\bar{k}_f=0$, $s^2(k_m)=\bar{k}_m=0$, and Eq. 3 reduces to $Ne_{(v)}=2N_t$

Case 2. If pollination occurs at random (no control on the number of pollen plants) [$s^2(k_m)=\bar{k}_m>0$] and k_f is controlled and equalized [$s^2(k_f)=\bar{k}_f=0$], then Eq. 3 can be expressed as

$Ne_{(v)}=2N_t/[1+\{s^2(k_m)/\bar{k}\}]=$
$Ne_{(v)}=2N_t/[1+(k_m)/\bar{k}\}]=$
$Ne_{(v)}=2N_t/[1+(1/2)\bar{k}/\bar{k}]=$
$Ne_{(v)}=(4/3)N_t$.

Case 3. If pollination is random [$s^2(k_m)=\bar{k}_m>0$] and different numbers of seeds are taken [$s^2(k_f)=\bar{k}_f>0$] (no control on male and female gametes), then Eq. 3 becomes

$Ne_{(v)}=2N_t/[1+\{\bar{k}_f+\bar{k}_m\}/\bar{k}]=N_t$
$Ne_{(v)}=2N_t/[1+\{(1/2)\bar{k}+(1/2)\bar{k}\}/\bar{k}]=N_t$.

When the accession to be regenerated is dioecious, that is, each plant can serve either as male or female, but not both, the effective size of the next generation is: (1) $N_e=8N_mN_f/(N_f+N_m)$ when the numbers of male and female parents are controlled (Hallauer and Miranda 1981). This occurs when k_m is controlled by plant-to-plant crosses, with each plant serving as a pollen or seed parent but not both; when $N_m=N_f=N$, $Ne=4N$; (2) $N_e=16N_mN_f/3(N_f+N_m)$ if only the number of female parents is controlled (but pollination is random); when $N_m=N_f=N$, $N_e=2.66N$; (3) $N_e=4N_mN_f/(N_f+Nm)$ when the km and kf are not controlled, i.e., pollination is random, and unequal numbers of seeds are secured per individual; when $N_m=N_f=N$, $N_e=2N$.

Practical procedures

As indicated by the preceding theoretical discussion, an optimal practical procedure for germplasm accession regeneration should control: (1) the number of pollen plants contributing male gametes to the next generation through controlled hand pollinations such as plant-to-plant crosses and chain crosses and (2) the number of seed plants contributing female gametes in the next generation, by taking equal numbers of seeds from each pollinated plant.

We will illustrate the concepts by discussing regeneration protocols developed for maize. A theoretically desirable regeneration procedure would involve planting 600 kernels (taken at random) in one isolated block. Six hundred random plant-to-plant crosses are then made, with each plant serving as male and female parents. The pollinated ears are harvested and equal quantities of kernels from each ear are saved and stored separately in the same packet for the next regeneration. In this case, the numbers of male and female parents are controlled so that the effective population size for the next regeneration will be $Ne_{(v)}=2N_t=1200$. With no control of the numbers of male parents contributing to the next generation, as in bulk pollination (bulking pollen from plants in one row to pollinate plants in a second row, resulting in bulk half-sib families) or with random (open) pollination, where equal numbers of seeds are selected from each pollinated ear, then $Ne_{(v)}=(4/3)N_t=800$. If the 600 plants are randomly pollinated and unequal numbers of seeds selected from each pollinated ear, then $Ne_{(v)}=N_t=600$. In these procedures the maize accession is treated as a monoecious species because each plant is used as both male and female. These regeneration procedures are very practical in field operations. However, seed mortality in the initial population often causes $s^2(k)>k$ and therefore $Ne_{(v)}< 2N_t$ or $Ne_{(v)}<(3/4)N_t$.

The following regeneration procedure may be superior to that just described. Assume that we have a maize accession consisting of 150 ears (half-sib families). Select, at random, two kernels from each of the 150 ears and place the 300 seeds in a packet. Repeat the procedure so that there are two packets of 300 seeds each. Plant one packet of 300 seeds in one block and make 150 random plant-to-plant crosses using each plant as a pollen parent or seed parent but not as both. Repeat the operation for the second packet of 300 seeds, producing another 150 random plant-to-plant crosses. Ears are harvested from one block and, if necessary, a total of 150 ears is secured by harvesting additional ears from the other block. For this procedure, the actual size of the breeding population is N=300 plants but N_e=1200 (Ne=4N) individuals. In this procedure the accession is treated as a dioecious species because each plant is used as male or female but not as both.

Crossa et al. (1993) recommended a sample size of 150–350 plants as required for capturing alleles at frequencies of 0.03–0.05, or higher, in each of 150 loci with a 90–95% probability. The probability of mating two plants in a block originating from the same ear is

$$P=(i-1)/\{(N)(i)-1\}$$

(where N is the number of original ears and i is the number of kernels taken from each ear). For example, for N=150 and i=2, P=0.003 (for i=1, P=0). If we have two rows of N*i plants each and we cross plants in one row with plants in the other row,

$$P=\{(N)(i)^2\}/\{(N)(i)^2\}=1/N$$

[where i^2 is the number of all possible crosses between sibs within a given family and $(N)(i)^2$ is the number of all possible crosses between rows].

Other practical options may be created by selecting more than two seeds per ear and making plant-to-plant crosses between paired but unrelated rows. If some of the rows are planted a few days later, crosses will involve a greater range of maturities.

For subsequent regenerations of a maize accession derived from 150 ears, seven packages with 450 seeds each (three seeds from each ear) can be prepared from seed produced in the first regeneration. The seventh package is used for germination tests. When the germination tests indicate the need for regeneration, 1–2 packages are planted in different blocks (450 plants per block). Paired crosses within each block are made such that at least 150 ears are harvested as before.

A less desirable option involves bulking equal numbers of seeds from each ear (e.g., 40–50 seeds) and planting a sample from the balanced bulk for regeneration. This option presents two main problems: first, if the sample selected from the bulk is not large enough, not all original ears (families) will be represented. For example, if equal numbers of seeds of 150 original ears are bulked and a sample of 100 seeds taken from the bulk, seeds from only 73 original families, on average, will be included in the sample (Crossa 1989). However, if 450 seeds are selected, seeds from 143 original families, on average, will be included in the sample. Second, even if the sample taken from the bulk is large enough to ensure that most original families are represented, this does not guarantee that each family will contribute the same number of female gametes. It is very likely that some ears (families) will contribute fewer seeds to the sample than others. The first deficiency could be overcome, to some extent, by taking more seeds (e.g., 600 seeds) from the bulk so that seeds from most original ears would be included. Then plant-to-plant crosses or chain crosses would be made at pollination with the result that more than 150 ears would be harvested, capturing alleles at frequencies of 0.03–0.05 or higher.

If the original collection was composed of kernels from 20 individuals or fewer, a severe genetic bottleneck may have occurred and some alleles at low frequencies (0.01–0.05) were probably lost. In this case, population size should be increased to 160–210 families or more before the germplasm is incorporated into the collection. This procedure would protect against further losses from genetic drift. Moreover, since the N_e over various generations is the harmonic mean of the N_es in different regeneration cycles, increasing N_e leads to an effective population size which is closest to the minimum requisite number. Therefore, an important aim in germplasm regeneration is to use the same (preferably large) number of individuals for different regeneration cycles.

The practice of preparing multiple samples (packets) for subsequent regenerations is appropriate for accession regeneration. In practice, a regeneration procedure that equalizes the number of progeny per family depends on the number of seeds representing each family. Furthermore, the number of seeds representing each original family will depend on seed viability and availability of land, labor, and management resources.

Further research is needed to compare the relative efficiency and cost effectiveness of different sample sizes in germplasm regeneration. Experiments underway at USDA/ARS-North Central Regional Plant Introduction Station and Iowa State University (Pollak and Millard, personal communication) are assessing the optimal sample size for testcross evaluation of maize germplasm and are determining the most cost efficient method of controlling pollination and its effects on allele frequencies in maize populations.

Practical aspects of forming a core subset

Core subsets are formed to facilitate germplasm use by providing ready access to the range of variation existing in the total germplasm collection of interest. Ready access to germplasm from a small representative subset can greatly facilitate preliminary evaluations. The remaining accessions will form a reserve subset that will be available for screening when a trait of interest is not found in the core subset.

Questions emerge related to the optimal procedure for identifying core subsets containing a maximum proportion of the existing genetic variability with a minimum of redundancy. Sampling strategies and methods for measuring and analyzing genetic diversity are important issues relating to core subset definition.

Brown (1989b) recommended stratified random sampling over simple random sampling for choosing accessions that will form the core subset. Simple random sampling is appropriate when the original collection is viewed without discontinuities, that is, without strata or groups. In this case, every accession in the collection has the same probability of being included in the sample. In stratified random sampling, the collection is subdivided into nonoverlapping groups, or strata, based on the accessions' characteristics or on their passport data, and a simple random sample is chosen within each final subgroup or substrata. Brown (1989b) suggested three strategies for determining how many accessions from each stratum to include in the core subset: (1) *Constant strategy*: choose equal numbers of accessions from each stratum; (2) *Proportional strategy*: choose a number of accessions from each stratum that is proportional to its frequency in the whole collection; (3) *Logarithmic strategy*: choose a number of accessions from each stratum that is proportional to the logarithm of the number of accessions in that stratum. The first strategy biases the core in favor of small strata, whereas the second strategy biases in favor of large ones.

How to form a core subset: an example

Wellhausen et al. (1951 1952) described and illustrated 25 races and three subraces of maize in Mexico. Additional

races have been described by Hernandez and Alanis (1976) and Sanchez and Goodman (1992) published additional information on relationships of Mexican races. A series of race bulletins for Latin America have been piblished by the National Academy of Sciences-National Research Council (NAS-NRC) and Goodman and Brown (1988) have summarized information on maize races. Wellhausen designated a few accessions within each race as "typical collections" which could be regarded as the first designation of a maize core subset.

CIMMYT's maize germplasm bank is now using a stratified random sampling strategy to update and form core subsets. The maize collection is first subdivided according to maize races. If races are represented by few accessions (fewer than 100), additional collections of the race may be required. Next, within each race, accessions having the same geographic origin are grouped by the region or elevation at which they were collected. Accessions for which these data are lacking could form an additional subgroup. Finally, a few (perhaps 5 to 15%) of the total accessions in each subgroup are selected to form the initial core subset based on distinctiveness and better agronomic traits. The core and reserve subsets can be regenerated using the methods described above and preserved in the bank's active collection.

The formation of a core subset of the maize race Tuxpeño (Taba et al. 1992; Crossa et al. 1994) exemplifies the process discussed above. In this case, the main goal was to form a core subset biased toward better agronomic types that maize breeders could use as a direct source of breeding germplasm. One problem is that some accessions could have been misclassified with respect to race. Racial assignments that are clearly incorrect affect the initial selection of the core subsets but these misclassifications can be identified and corrected by performing a cluster analysis over races after the preliminary maize core subset has been developed.

The next step in forming a core collection is stratified sampling. We have stratified the collection according to the following classification criteria: (1) the particular maize race, in this case, Tuxpeño, (2) the geographic location where the accessions (or accession composites) were collected, and (3) the cutting point applied to the dendrogram obtained from classification analysis (cluster analysis). This stratum comprises several homogeneous substrata (subgroups) of 2–5 accessions or accession composites each. One or two accessions or accession composites are randomly selected from each homogeneous substratum (Crossa et al. 1994). This process resembles the stratified random sampling procedure proposed by Brown (1989b). However, in this case, the final stratum is deternined by the cutting point used on the dendrogram derived from a cluster analysis on morphological and agronomic data. This may cause atypical or badly classified accessions to be over-emphasized.

From a total of 848 Tuxpeño accessions and accession composites, 175 were selected based on lodging and adaptation (Taba et al. 1992) assessed from multiple location trials. Some of the Tuxpeño accessions are a combination of accessions and accession composites; compositing accessions tends to centralize the composite mean and changes the probabilities of various subsets. Although Wellhausen et al. (1952) identified only a few typical Tuxpeño accessions, most of them are represented in the accession composites included in this study.

The second classification criterion was ecogeographical regions: 58 of the 175 accessions were collected in *dry-ecology* regions under 1000 meters above sea level, and 48 of the 175 come from *wet-ecology* areas below 1000 meters above sea level. The remaining 69 are classified as *mixed-ecology* accessions, or accession composites, and were collected in both dry-ecology and wet-ecology regions. All 175 accessions or accession composites were planted in a replicated trial at two locations in Mexico. Data on various agronomic and morphological traits and grain yield were recorded. The third classification criterion was derived from a two-stage statistical analysis: (1) a classification study (cluster analysis) to determine whether the accessions and accession composites could be regarded as consisting of distinct groups; and (2) an ordination study (principal components analysis) to examine spatial relationships among accessions and accession composites. Classification and ordination were performed on pairs in a step-wise manner and results of cluster analysis: (1) served to define small, homogeneous substrata from which accessions or accession composites are randomly selected and (2) were compared to results obtained from principal component analysis.

The first step involves performing cluster analysis and principal component analysis on all 175 accessions and accession composites. The dendrogram (data not shown) resulting from cluster analysis served as a basis for selecting accessions or accession composites within each substratum. As mentioned above, the third classification criterion considers the cutting point of the dendrogram, which was based on the utility of the subgroups resulting from it. Crossa et al. (1994) were conservative and used small subgroups from the dendrogram so that accessions and accession composites within subgroups were homogeneous; one or two accessions or accession composites were randomly selected from each subgroup. A total of 80 accessions and accession composites was selected using this procedure.

Results from cluster analysis were compared with those obtained from principal component analysis on all 175 Tuxpeño accessions and accession composites. Placement of 175 accessions and accession composites on the first two principal component axes indicated that dry-ecology and wet-ecology Tuxpeño accessions and accession composites had distinct agronomic and morphological traits (Fig. 1). Tuxpeño accessions and accession composites in the mixed-ecology category were placed between the other two subgroups. The first three principal components accounted for 60%, 14%, and 11% of total variability, respectively.

The 80 accessions and accession composites (26 dry-ecology, 30 wet-ecology, and 24 mixed-ecology) selected with cluster analysis were advanced to the next step, which involved conducting cluster analysis and principal compo-

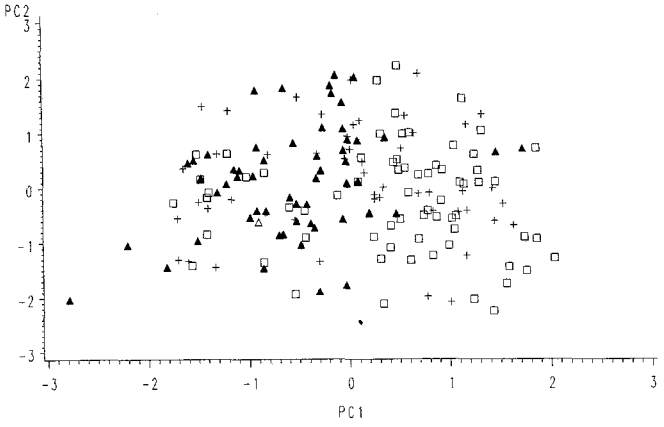

Fig. 1 Plot of the first two principal component axes of 175 Tuxpeño accessions. *Black triangles* represent dry-ecology accessions, *white squares* wet-ecology accessions, and *plus signs* mixed-ecology accessions (from Crossa et al. 1994)

nent analysis on the 80 previously selected accessions and accession composites. The dendrogram from cluster analysis on the 80 accessions and accession composites is shown in Fig. 2. The last two main groups in the dendrogram represent the two major groups of accessions. One main group comprises 21 accessions and one accession composite (C.TUX V) (14 wet-ecology and eight mixed-ecology), and the other includes all 26 dry-ecology accessions plus the remaining wet-ecology and mixed-ecology accessions (two accession composites were included, C. AZT.T and C.TUXPEN). As in the first step, the final cutting point was set further down into the branches of the dendrogram so that less diverse substrata of accessions and accession composite were found. One or two accessions or accession composites were randomly selected from each subgroup, and 45 accessions and three accession composites were selected to form the final core subset.

Principal component analysis of the 80 accessions and accession composites separated the two major groups (wet-ecology and dry-ecology); mixed-ecology taxa overlapped these groups (Fig. 3), and confirmed the results obtained from cluster analysis. A plot of the first two principal component axes showed the diversity of the core subset (48 accessions and 3 accession composites) (19 wet-ecology, 13 dry-ecology, and 16 mixed-ecology) based on morphological and agronomic characteristics (Fig. 4). It is expected that these 48 accessions will preserve rare and widespread alleles that were present in the original Tuxpeño race collection at frequencies of 0.03 to 0.05 or higher (Crossa et al. 1993).

When data on morphological and agronomic attributes are available, the procedure outlined above may be appropriate for selecting accessions for the core subset. Stratified random sampling, using the cutting point of the dendrogram as a classification criterion, exploits fully the data used in the analysis. This takes into consideration similarities (or dissimilarities) among accessions across their morphological and agronomic attributes. Accessions and accession composites from each small dendrogram substratum have the same probability of being included in the final core subset, provided they are selected at random. However, in some instances there may be reasons not to choose accessions randomly. For example, some accessions have been through fewer "bottlenecks" (regenerations) and some may have greater characterization or evaluation information (cytology, ecology, breeding) than others. Some may be farmer accessions or market accessions; some may have no passport data.

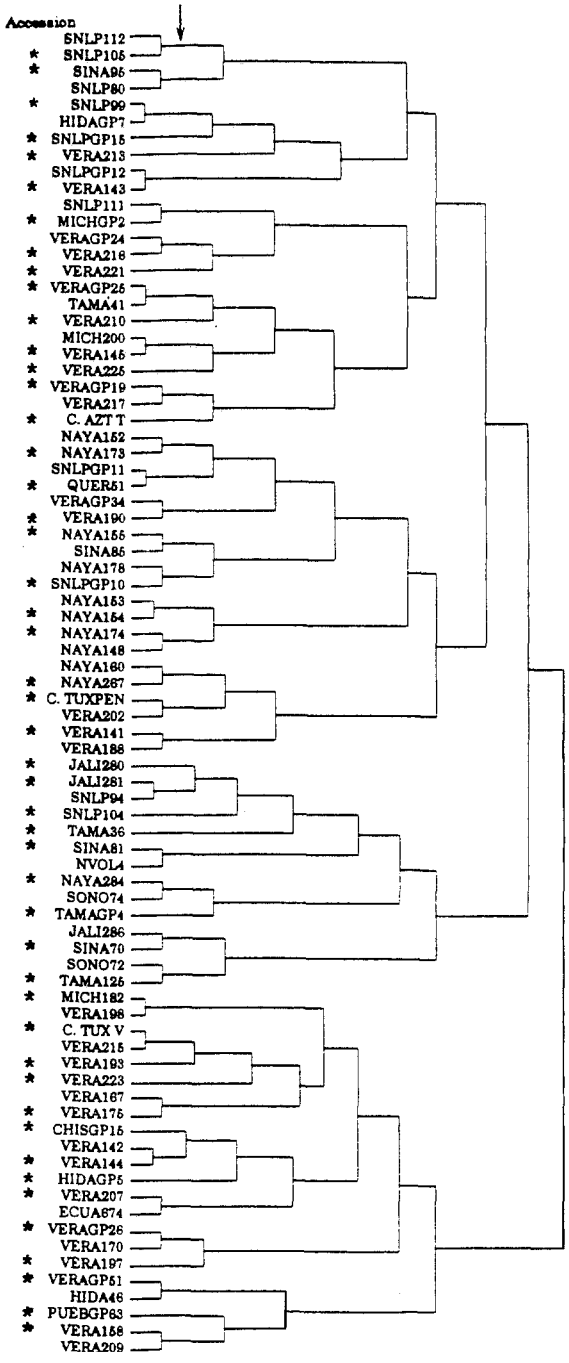

Fig. 2 Dendrogram from a cluster analysis of 80 Tuxpeño accessions. The 48 accessions selected to form the core subset are marked with an *asterisk*. The *arrow* marks the cutting point (from Crossa et al. 1994)

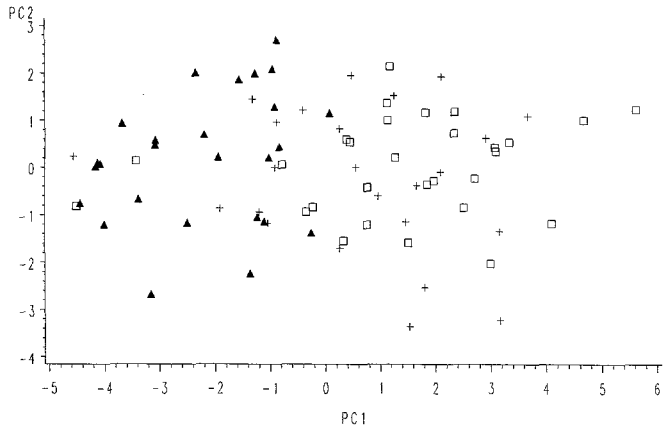

Fig. 3 Plot of the first two principal component axes of 80 Tuxpeño accessions. *Black triangles* represent dry-ecology accessions, *white squares* wet-ecology accessions, and *plus signs* mixed-ecology accessions (from Crossa et al. 1994)

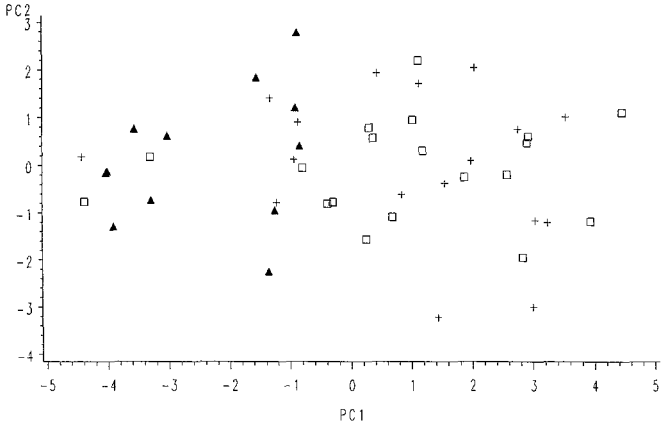

Fig. 4 Plot of the first two principal component axes of 48 Tuxpeño accessions. *Black triangles* represent dry-ecology accessions, *white squares* wet-ecology accessions, and *plus signs* mixed-ecology accessions (from Crossa et al. 1994)

Concluding remarks

Characterization and evaluation of core subsets will make germplasm more useful to plant breeders and molecular biologists. Preliminary evaluation of core subsets should involve measuring resistance to important biotic and abiotic stresses, as well as other agronomic traits such as yield. It will usually be necessary to retain the geographical subgroup structure for this evaluation because adaptation varies according to elevation and latitude.

Possible loss of genetic diversity and drastic changes in allelic frequencies when forming core subsets should be confirmed by both biochemical and genetic methods (such as allozyme analysis). Characterizing germplasm in the initial core subset by means of molecular markers (RFLPs, RAPDs or isozymes) would provide additional information that, together with morphological, geographic and agronomic data, may help to assess genetic diversity and modify the core subset.

Genetic resources can be a source of resistance to newly evolved pathogen strains and insect biotypes as they arise over time (Shands and Wiesner 1991 1992). No matter how thorough the germplasm is characterized or evaluated, its intrinsic value may be unknown before an outbreak of disease or insects actually occurs. Hence, germplasm banks should maintain germplasm collections which preserve coadapted alleles. Representative samples of landraces from all primary and secondary centers of origin must be conserved because they have accumulated mutations for millenia. Therefore an optimal core subset for each crop would comprise landraces from the various geographical sub-regions and perhaps some improved cultivars and representative private and public elite germplasm.

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