Quantitative Methods for Sampling of Germplasm Collections

Getting the best out of molecular markers when creating core collections

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Thomas L. Odong

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To my father, Mr. Kamilo Oyaro Orik (RIP) and my mother Balbina Atyang Oyaro. Father you always took pride in my academic success, it is a pity that you did not live to see this day; mum (oma) we are what we are today because of the sacrifices that you made.

Contents

Chapter 1

General Introduction

1.1 Background

Ex-situ germplasm collections have increased enormously in number and size over the last three to four decades as a result of global efforts to conserve plant genetic resources for food and agriculture. Globally, over seven million accessions of different crop species are conserved in about 1750 genebanks (Upadhyaya et al. 2010). These accessions are of a diverse nature and include landraces, selected lines from landraces, elite breeding lines, released varieties, wild and weedy relatives of cultigens, and genetic stocks from different areas of origin. Because of this diverse nature, they can provide all relevant allelic diversity necessary for plant improvement. However, the large sizes of these collections hinder full exploitation of all available genetic resources. The idea of picking an accession with genes of interest from say a collection of 80,000 rice accessions is simply mind boggling for a breeder and this is one of the reasons that the potentials of plant genetic resources in genebanks have remain largely unexploited. The approach of forming core collections (core sub-sets) was introduced to ensure efficient and effective management and utilization of all accumulated plant genetic resources. Frankel (1984) defined a core collection as a limited set of accessions representing, with minimum repetitiveness, the genetic diversity of a crop species and its wild relatives. The idea of core collections is a radical departure from first generation genetic resource conservation thinking which stresses accumulation without much concern about utilization. From the original definition, several operational definitions have since been coined (see Brown, 1995; van Hintum et al. 2000).

Core collections have many roles to play in the management and use of genetic resources. Genebank curators have the responsibility for conservation, regeneration, safety duplication, documentation, evaluation and characterisation of the genetic resources in their collections. These activities of the genebank often require the curators to make choices or set priorities among accessions because of limited resources (Brown, 1995). Because a core collection is smaller in size compared to the whole collection, it enables some operations of the genebank, such as evaluation, to be handled more efficiently and effectively. The limited size of a core is key to its manageability, and in many cases the representation of the collection's diversity enables the core to function as a reference set of accessions for the whole collection (Brown and Spillane, 1999). On the other hand, having a small sample of accessions (core collection) representing the diversity exhibited by a crop species coupled with evaluation or characterization data would greatly encourage the breeders to effectively exploit the potential of these genetic resources.

Since the inception of the idea of core collections almost three decades ago, a vast body of literature on the theory and practice of core collections has accumulated. Very many approaches for selecting core collections have been proposed and used (*e.g*. M-Strat (Gouesnard et al. 2001), Genetic distance sampling (Jansen and van Hintum 2007), PowerCore (Kim et al. 2007) and Core Hunter (Thachuk et al. 2009)). For several plant species, core collections have been established using different approaches: sweet potato (Huaman et al. 1999), maize (Malosetti and Abadie 2001), chickpea (Upadhyaya et al. 2001), peanut (Upadhyaya et al. 2002), rice (Li et al. 2002), soybean (Wang et al. 2006), bread wheat (Balfourier et al. 2007) and Chilean common bean (Mario et al. 2010). However, several challenges still exist when it comes to making decisions on methodologies for selection of core collections.

Designation of a core collection involves a number of decisions especially on quantitative sampling methodologies. The key issues include amongst others: a) choice of the size of core collection b) determination of the genetic structure of germplasm collections (stratification/grouping) c) determination of the number of accession to be selected from each group d) method to select accessions from the different groups and e) evaluating the quality of core collections. Each of the key issue mentioned above have received research attention to a varying degree but a lot still need be done. In the following section we give brief descriptions of the challenges that motivated the different aspects of the research that led to this thesis.

1.2 Determination of the genetic structure of germplasm collections

Determination of the genetic structure (partitioning) of heterogeneous germplasm collections is an essential component of the sampling of core collections. Partitioning of germplasm collections before sampling ensures that both the genetic and the ecological spectra of germplasm collections are fully represented in core collections (Brown 1995; van Hintum et al. 2000). In addition, even in cases where core collections were selected without stratification it may be necessary to associate an accession in the core collection with accessions in the entire collection; this association can be based on the group structure of the germplasm collection. The determination of genetic structure is also an important aspect of association studies (Wang et al. 2005; Shriner et al. 2007); general agreements exist among researchers that incorporating population structure into statistical models used in association studies is necessary to avoid false positives (Pritchard et al. 2000b; Flint-Garcia et al. 2003; Zhu et al. 2008).

Whether the genetic structure is needed for use in sampling core collections or for association studies, an important challenge still is the choice of a method for determining the genetic structure. In the past, determination of the genetic structure of germplasm collections has mainly been done using passport data (van Hintum 2000) or multivariate statistical methods such as cluster analysis, principal component analysis, and multidimensional scaling, usually based on agronomic data (Peeters and Martinelli 1989; Franco et al. 1997, 2005, 2006). However, in recent years, many new methods have been developed especially for studying the genetic structure of natural populations using molecular markers, e.g. STRUCTURE (Pritchard et al. 2000), PCA (Patterson et al. 2006) and PCO-MC (Reeves and Richards 2009). Despite the introduction of these approaches, most researchers in the plant sciences still use traditional methods especially hierarchical clustering techniques for studying genetic diversity in crop species (see Folkertsma et al. 2005; Perumal et al. 2007; Barro-Kondombo et al. 2010; D'hoop et al.

2010). The popularity of traditional hierarchical clustering techniques such as Ward's method stems from the fact that they a) require little computer time compared to other methods, b) are available in many general statistical packages, c) are frequently used in different types of applications and d) the output is easy to interpret. Moreover, traditional hierarchical clustering techniques do not require genetic assumptions such as Hardy-Weinberg or linkage equilibrium. However, with the changes in types, quality and quantity of data used for studying genetic structure of germplasm collections, the performance of traditional hierarchical clustering techniques ought to be evaluated. For example, most evaluations of the performance of hierarchical clustering methods were based on data sets of very limited sizes (Milligan and Cooper 1985). In addition, most studies carried out to evaluate the performance of hierarchical clustering methods with respect to germplasm collections were not carried out molecular marker data (Peeters and Martinelli, 1989; Franco et al. 1997, 2005, 2006). Currently, we are not aware of any study in which the performance of hierarchical clustering techniques was evaluated specifically using molecular marker data. With the expected reduction in the cost of genotyping, researchers will be faced with datasets of thousands of accessions genotyped with many molecular markers so there is strong need to evaluate the performance of the traditional hierarchical clustering techniques using large sets of molecular marker data. In general it is not clear how traditional clustering will perform under different factors affecting genetic diversity like migration and reproductive system of the materials that constitute germplasm collections. The response received on a recent paper (Odong et al. 2011; Chapter 2) on cluster analysis using molecular markers is a good indication of the growing interest of researchers in this topic. This paper was consistently the most downloaded paper from Theoretical and Applied Genetics for a period seven months (April - November 2011) with over 300 downloads per month. In addition, it has been suggested in the literature (Patterson et al. 2006) that the use of principal component analysis (PCA) could boost the performance the traditional clustering technique for determining the population genetic structures. The integration of PCA and cluster analysis is likely to contribute tremendously to improving the ability of the traditional cluster analysis when determining the genetic structure of germplasm collections (Chapter 3).

1.3 Connecting germplasm collections in different genebanks : Reference sets of accessions and molecular markers

The exploitation of the full potential of plant genetic resources cannot be complete without linking information on genetic diversity from the different germplasm collections (genebanks) around the world. It is possible to establish relations between genebank collections by defining for each crop a small but informative set of accessions, together with a small set of reliable molecular markers, that can be used as reference material (reference sets). The reference material should be an adequate representation of the genetic diversity of that crop as stored in genebanks around the world. In that case, molecular marker information can be used to place new accessions in the spectrum of current accessions. The designation of reference sets will help in the identification of overlaps between germplasm collections and this will allow these collections to be analyzed together thus enlarging the space of our inference. The reference sets can also be used to connect different population genetic and quantitative genetic studies, including association studies. However, defining statistical methods for selecting such a representative subsets of accessions and molecular markers is a challenge. The Generation Challenge Programme –CGP (GCP; http://www.generationcp.org) initiated the process of constructing reference sets by genotyping large numbers of accessions of important agricultural crops using microsatellite markers.

For the selection of such a representative subset of accessions, the ideal method should be based on the relationship between the selected accessions (entries) and the accessions not selected in the subset. Most existing algorithms for selection of core collection (MSTRAT (Gouesnard et al. 2001), PowerCore (Kim et al. 2007) and Core Hunter (Thachuk et al. 2009)) pay more attention to the content of the core collections but tend to ignore the relationships between the selected entries and those accessions not included in the subset. In addition, by aiming at maximizing genetic diversity parameters such as allelic richness, average distances between selected accessions, methods such as MSTRAT (Gouesnard et al. 2001) are likely to select mainly non-representative accessions (“outliers”). In other words, none of the existing algorithms for selecting core collections was developed to select accessions to serve as representatives around which the other accessions can be positioned.

For the selection of a subset of molecular markers, the aim is to obtain a subset of markers that would preserve the major population genetic structure in the data. Currently the most common criterion used for selection of molecular markers in plant germplasm studies is the polymorphic information content – PIC (Botstein et al. 1980). It should be noted that PIC favors molecular markers with very many alleles of equal frequencies. Although molecular markers with high PIC may be good for differentiating between individual accessions, those markers are likely to perform poorly with respect to detecting differences between groups (population structure). In addition, two markers with high PIC may contain similar information and thus introduce redundancy in the subset of selected markers. Consequently, there is a need to come up with methods for the selection of subsets of molecular markers which describe the major genetic structure in the data with minimum redundancy.

1.4 Quality criteria for evaluation of core collection

When comparing the options for assembling core collections, one of the challenges is to choose the right evaluation criteria for gauging the quality of the result. Various criteria for determining the suitability of a core collection have been suggested in the literature, yet very little attention has been given to the analysis of these quality criteria. In fact most researchers appear to choose quality evaluation criteria simply because they were used in earlier publications. There is a need to clearly define criteria for the evaluation of the quality of core collections and to determine the conditions under which these criteria are suitable. For example, a core subset formed for the purpose of capturing rare or extreme traits (e.g. high resistance to pest or high yield) should be evaluated differently from one formed with the intention of representing the pattern of genetic diversity in the collection.

1.5 Study objectives and outline of the thesis

The work in this thesis aims at improving knowledge associated with the sampling of core collections and the roles that core collections have to play in the utilization of plant genetic resources. This thesis looked at three key aspects of core collection development and its roles in utilization of plant genetic resources: a) determination of the genetic structure of germplasm collections and the relevance of the genetic structure in core selection and utilization of germplasm resources in general b) creating links between genetic resources stored in different parts of the world and c) critical examination of criteria for evaluating the quality of core collections.

In chapter 2 we study the appropriateness of traditional hierarchical clustering techniques (Ward's method and UPGMA) for determining the structure of germplasm collections using molecular marker data. The relationships between criteria used for evaluating the output of cluster analysis (co-phenetic correlation coefficient and agglomerative coefficient) and population genetic structure parameters (*F*-statistic) will be explored. The performance of hierarchical clustering techniques will be compared amongst themselves and with STRUCTURE (Pritchard et al. 2000). STRUCTURE is a computer program especially developed for studying the population structure of natural populations. Real and simulated data sets were used in the study.

Chapter 3 we look at the possibilities of using principal component analysis (PCA) to boost the performance of traditional hierarchical clustering techniques for determining the genetic structure of germplasm collections. In this chapter we will study the ability of the Tracy-Widom distribution to accurately determine the number of genetically differentiated groups in germplasm collections. The significant principal components (PCs) based on the Tracy-Widom distribution will be used for the grouping of accessions usinga traditional hierarchical clustering technique (Ward's method) and a model-based clustering method (Mclust). The performance of Ward's clustering technique using Euclidean distance based on significant PCs (reduced data set) will be compared with clustering based on several other distances measures calculated using the full data set.

In chapter 4 we propose and discuss several statistical techniques for defining a representative subset of accessions and molecular markers that can be used for connecting genetic resources in different genebanks. We will study Genetic Distance Optimization (GDOpt) as a suitable method for the selection of a representative set of accessions. For the selection of molecular markers we will evaluate backward elimination methods as well as methods based on principal component analysis. The current practice of using the polymorphic information content (PIC) as a criterion for selecting molecular markers will be used as a baseline against which the other methods will be compared.

Chapter 5 we critically examine criteria for quality evaluation of core collections. We will define different types of core collections and relate each type of core collection with suitable quality evaluation criteria. We propose distance-based evaluation criteria and evaluated their performance using real data sets.

Finally chapter 6 provide a general discussion and draw conclusions.

Chapter 2

Determination of genetic structure of germplasm collections: Are traditional hierarchical clustering methods appropriate for molecular marker data?

Abstract

Despite the availability of newer approaches, traditional hierarchical clustering remains very popular in genetic diversity studies in plants. However, little is known about its suitability for molecular marker data. We studied the performance of traditional hierarchical clustering techniques using real and simulated molecular marker data. Our study also compared the performance of traditional hierarchical clustering with model-based clustering (STRUCTURE). We showed that the co-phenetic correlation coefficient is directly related to subgroup differentiation and can thus be used as an indicator of the presence of genetically distinct subgroups in germplasm collections. Whereas UPGMA performed well in preserving distances between accessions, Ward excelled in recovering groups. Our results also showed a close similarity between clusters obtained by Ward and by STRUCTURE. Traditional cluster analysis can provide an easy and effective way of determining structure in germplasm collections using molecular marker data, and, the output can be used for sampling core collections or for association studies.

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2.1 **Introduction**

Information about the structure of germplasm collections is of great importance for both the conservation and utilization of genetic resources collected in genebanks. Because of the diverse nature of genebank germplasm materials (landraces, selected lines from landraces, elite breeding lines, released varieties, wild and weedy relatives of the cultigen, and genetic stocks from different areas of origin), they provide all relevant allelic diversity necessary for plant improvement. These materials are therefore very suitable for example for association studies (D'hoop et al. 2010). However, the large numbers of accessions accumulated in genebanks reduce the efficiency and effectiveness with which these genetic resources can be exploited. The approach of forming core collections (core sub-sets) was introduced to solve the above problem. Frankel (1984) defined a core collection as a limited set of accessions representing, with minimum repetitiveness, the genetic diversity of a crop species and its wild relatives. Determination of the genetic structure (partitioning) of heterogeneous germplasm collections is an essential component in the sampling of core collections since partitioning of germplasm collections before sampling ensures that both the genetic and the ecological spectra of germplasm collections are fully represented in core collections (Brown 1995; van Hintum et al. 2000). In addition, it may be necessary to associate a accessions in the core collection with the entire collection; the association can be based on the group structure.

The determination of genetic structures of germplasm collections is also an important aspect of association studies (Wang et al. 2005; Shriner et al. 2007). General agreement exist among researchers that incorporating population structure into statistical models used in association mapping is necessary to avoid false positives (Pritchard et al. 2000b; Flint-Garcia et al. 2003; Zhu et al. 2008). The general model for association mapping can be written as "*phenotype = marker + genotype + error",* and test for a marker effect is equivalent to testing for a QTL. Typically genotype is a random factor whose effects are structured by kinship or population structure. This simple model can be improved by incorporating information on the relationships between the genotypes a.k.a. population structure. The relationship between phenotype and marker can be tested within the different groups (e.g. Remingston et al. 2001; Simko et al. 2004) or genetic groups can be used as an extra factor or as a covariate in modelling the relationship (*e.g*. Thornsberry et al. 2001; Wilson et al. 2004). Yu et al. (2006) went further by introducing a mixed model approach which incorporates both population structure (Q) and kinship (K) in modelling the relationship between phenotype and marker. Another important method for incorporating population structure in association studies involves the use of principal components (Price et al. 2006).

Whether the genetic structure is needed for use in sampling core collections or for association studies, an important challenge still is the choice of a method for determining the genetic structure of germplasm collections. In the past determination of the genetic structure of germplasm collections has mainly been done using traditional multivariate statistical methods such as cluster analysis, principal component analysis, and multidimensional scaling, usually based on agronomic data (Peeters and Martinelli 1989; Franco et al. 1997, 2005, 2006).

In recent years, many new methods have been developed especially for studying structure in natural populations using molecular markers, *e.g*. STRUCTURE (Pritchard et al. 2000a), PCA (Patterson et al. 2006) and PCO-MC (Reeves and Richards 2009). These methods can also be used for studying genetic structure in germplasm collections. However, traditional hierarchical clustering is still a very popular method for studying genetic diversity in crop species (see D'hoop et al. 2010; Barro-Kondombo et al. 2010; Perumal et al. 2007; Folkertsma et al. 2005). Its popularity stems from the fact that it requires little computer time compared to other methods, it is available in many general statistical packages, it is frequently used in different types of applications and it is easy to understand. Moreover, it does not require genetic assumptions such as Hardy-Weinberg or linkage equilibrium. Hierarchical clustering requires decisions about the distance measure, the clustering algorithm and the evaluation of dendrograms, amongst others. Most evaluations of the performance of hierarchical clustering methods were based on data sets of limited size (Milligan and Cooper 1985). In addition, most studies carried out to evaluate the performance of hierarchical clustering methods with respect to germplasm collections were on non-molecular marker data (Peeters and Martinelli, 1989; Franco et al. 1997, 2005, 2006). We are not aware of any study in which the performance of hierarchical clustering techniques were evaluated specifically using molecular marker data. With the expected reduction in the cost of genotyping, we will be faced with datasets of thousands of accessions genotyped with several molecular markers so there is strong need to evaluate the performance of the traditional hierarchical clustering techniques using large sets of molecular marker data. The structure of genetic diversity in germplasm collections is totally different compared to natural populations. It is not clear how traditional clustering will perform under different factors affecting genetic diversity like migration and reproductive system of the materials that constitute germplasm collections. As pointed out by Mohammadi (2003), very few studies in plant genetic diversity have critically analyzed the performance of different clustering procedures especially with respect to molecular markers.

Several methods for evaluating the results of hierarchical clustering techniques exist. When performing hierarchical cluster analysis, we are interested in answering some of the following questions: 1) is there agreement between the original distances and the distances between individuals as represented by the dendrogram 2) what can the dendogram tell us about structure in the data set and 3) what is the optimum number of clusters for a given data set? One of the most popular measures of agreement between the original distances and the distances in dendrogram is the co-phenetic correlation coefficient (CPCC) (Sokal and Rohlf 1962); another measure is the stress criterion of Kruskal (1964). Only a few measures for the presence of hierarchical structure can be found in the literature. Kaufman and Rousseeuw (1990) proposed the agglomerative coefficient (AC) as a criterion for measuring the amount of hierarchical structure in the data. A large number of methods have been proposed to deal with the optimum-number-of-clusters problem. A classical study is that of Milligan and Cooper (1985) who examined the performance of 30 of such criteria. Since then many criteria for determining the optimal number of clusters were introduced: the silhouette statistic (Rousseeuw 1987), Krzanowski and Lai's index (Krzanowski and Lai 1988), the gap method

(Tibshirani and Walther 2001), the Clest method (Dudoit and Fridlyand 2002), the jump method (Sugar and James 2003) and the weighted gap method (Yan and Ye 2007). In general, little attention has been paid to the behaviour of the above measures and methods in relation to molecular marker data from germplasm collections. A literature search indicated that so far no study tried to relate the amount of genetic structure in a germplasm collections to the performance of hierarchical cluster analysis techniques. The main objective of our study is to determine a relationship between dendogram evaluation criteria such as CPCC, AC to subgroup differentiation (genetic structure). In addition, we also compared the performance of hierarchical clustering techniques with model-based clustering methods.

In this paper, the merits of hierarchical clustering techniques for application in germplasm collections will be considered. The materials and methods section contains a brief description and overview of clustering techniques, the evaluation criteria and the methods used for generating simulated data. The real data set used for illustration in this paper is also described. In the results section, we present results of cluster analysis of both real and simulated data sets. We compare the results of two traditional hierarchical clustering techniques (UPGMA and Ward) with the model-based cluster analysis program STRUCTURE (Pritchard et al. 2000a), and show using simulated data how different evaluation criteria of hierarchical cluster analysis are related to subpopulation differentiation.

2.2 Material and Methods

2.2.1 Motivation of the study

This study was motivated by the need to study genetic diversity of several important food crops under the Generation Challenge Programme-GCP (www.generationcp.org). The Generation Challenge Programme is a broad network of partners from international agricultural research institutes and national agricultural research programs collectively working to improve crop productivity in the developing world, especially environments prone to drought, low soil fertility, pests and diseases. All the real data sets used in this study were generated under GCP subprogram I – Crop Genetic Diversity.

2.2.2 Data

Real data: The real data that will be used to illustrate methods consist of 1014 accessions of coconut (*Cocos nucifera*) genotyped with 30 SSR markers. The accessions were collected from different regions of the world: West Africa (32), North America (52), South Asia (62), Latin America (72), Central America & the Caribbean (109), East Africa (124), South East Asia (183) and the Pacific Islands (380). Coconut is a diploid, mainly out-crossing species. Most of the accessions in this collection were indicated as tall; 43 dwarf accessions were present mainly from South East Asia. Dwarf coconuts have a high degree of self-fertilization. Because of its usefulness, coconut has been extensively distributed around the world. For this study, the coconut data were selected because it contained larger numbers of accessions of each of the diverse origins (a typical genebank germplasm collection).

Two additional data sets, on potato (*Solanum* species) and common bean (*Phaseolus vulgaris*), are described, analyzed and discussed in Appendix 1. The potato data (233 accessions; 50 SSR markers) contained several unique accessions which act like outliers. All accessions used in this study are diploid. Unlike coconut and potato, common bean is a predominantly selfing species. The common bean data (603 accessions; 36 SSR markers) consist of accessions of two distinct types, Mesoamerican and Andean.

Simulated data Marker data were simulated by SimuPOP (Peng and Kimmel 2005), a forward-time population genetic simulation environment. We used a finite island (Wright, 1931) and a stepping stone (Kimura, 1953) migration models. In each generation, random mating (with 2% selfing) was assumed to produce a diploid genotype for 30 unlinked loci for each individual, which had a certain probability of migrating to another subpopulation. We simulated 1000 individuals in ***five*** subpopulations of varying subpopulation differentiation levels (differentiation between subpopulations was determined by migration rates and number of generations). The migration rates used in this study were 0, 1 and 2 migrants per subpopulation. At each of the 30 loci, the average allele frequency of coconut data was used as the starting allele frequency for the simulation. Within each parameter set, all the loci had the same mutation dynamics, which occurs according to a K-allele model (KAM). Under the KAM model, there are K possible allelic states, and any allele has a constant probability of mutating into any of the other K–1 allelic states (Crow and Kimura 1970). A mutation rate of 2 x 10^{-5} with 50 possible allelic states was used in the simulation.   The mutation parameters were set to mimic highly polymorphic markers such as SSR markers. However, in this case the role of mutation is very limited since we used a limited number of generations in the simulation. In addition to using alleles from real data as starting frequencies for simulation, the numbers of generations for the simulations were restricted (from 5 to 200 generations) to mimic the situation of agricultural crops in the genebanks.

2.2.3 Distance

In this paper, we used genetic distances (*D*) based on the proportion of shared alleles (*PSA*) where

D = 1 - *PSA*, and

$$PSA = \left[\sum_{m=1}^{M} \sum_{a=1}^{A_m} \min(f_{1ma}, f_{2ma}) \right] / M \ ,$$

where in diploids f_{1ma} and f_{2ma} are the frequencies of allele a (a=1, 2… A_m ; A_m is the total number of alleles for molecular marker m (m=1, 2… M)) in individuals 1 and 2, respectively, and $f_{1ma}, f_{2ma} = 0, \frac{1}{2} \text{or} 1$. For more information on the proportion of shared alleles as similarity measure, see Bowcock et al. (1994), Chakraborty and Jin (1994) and

Chang et al. (2009). The effect of distance measures on the grouping of accessions will be considered in another paper.

2.2.4 Clustering Techniques

Hierarchical clustering techniques From the literature on determination of the structure of plant germplasm collections, the most popular clustering methods are Unweighted Pair Group Method with Arithmetic Mean (UPGMA; (Sokal and Michener 1958)) and Ward's method (Ward 1963). For the purpose of this study, only these two hierarchical clustering methods (hereafter referred to as UPGMA and Ward) will be discussed; both methods are well described in Kaufman and Rousseeuw (1990) and Johnson and Wichern (2002).

The differences between hierarchical clustering algorithms lie mainly in how the distances between pairs of objects or clusters are defined. In UPGMA the distance between two clusters is defined as the unweighted mean of the distances between all pairs of accessions, one from each cluster. At each step, the two nearest clusters are joined. Ward employs analysis of variance (ANOVA) approach for calculating the distances between clusters. For each pair of clusters, the sum of squared deviations between each accession and the centre of the new cluster (error sum of squares) is calculated and the pair of clusters that yields the lowest error sum of squares are merged. In other words at each step in the clustering process, the effect of the union of every possible pair of clusters is considered, and the two clusters that produce the smallest increase in error sum of squares are joined. It should be noted that both UPGMA and Ward use Lance and William's recurrence formula (Lance and Williams 1967) to operate directly on any distance matrix.

Model-based clustering techniques The most popular model-based clustering technique is STRUCTURE (Pritchard et al. 2000a; Falush et al. 2003, 2007). STRUCTURE assumes a model with K populations; K may be unknown. It is assumed that within populations loci are in linkage equilibrium and Hardy-Weinberg equilibrium; STRUCTURE assigns individuals to populations to achieve this.

Evaluation Criteria

Co-phenetic Correlation Coefficient The Co-phenetic Correlation Coefficient (CPCC) is a product-moment correlation coefficient between co-phenetic distances and distance matrix (input distance matrix) obtained from the data. The co-phenetic distance between two accessions is defined as the distance at which two accessions are first clustered together in a dendrogram going from the bottom to the top. The CPCC therefore measures the relationships between the original pair wise distance between accessions (true distances) and pair wise distances between accessions predicted using the dendogram. Farris (1969) proved algebraically that among the traditional hierarchical clustering algorithms, UPGMA always produces the highest CPCC; earlier this was shown empirically by Sokal and Rohlf (1962).

Agglomerative Coefficient The Agglomerative Coefficient (AC) described by Kaufman and Rousseeuw (1990), is one of the methods proposed for quantifying hierarchical structure. The agglomerative coefficient is defined as

$$AC = 1 - \frac{d_{average}}{d_{final}},$$

where $d_{average}$ denotes the average distance at which each object merges with one or more objects for the first time, d_{final} is the distance at which all the objects are merged into one cluster. It is clear from the formula that AC is highly affected by the distance (d_{final}) at the final merger of the algorithm i.e. as long as the value of d_{final} is high relative to $d_{average}$, AC will always be close to one. The use of AC in plant diversity studies is quite limited but it has been used in other fields.

Determining the optimal number of clusters

Milligan and Cooper (1985) evaluated 30 rules for determining the optimal number of clusters. For illustration, one of the best six methods according to Milligan and Cooper (1985), the point biserial correlation, will be compared with the average silhouette coefficient proposed by Rousseew (1987). The two criteria were chosen because of their easy interpretation. The Point-Biserial Correlation (PBC) (Milligan 1981) is defined as the correlation between corresponding entries in the original distance matrix and a matrix consisting of zeros and ones indicating whether two objects are in the same cluster or not. This is an easy measure of the resemblance between the distance matrix and the resulting tree.

The Average Silhouette Coefficient (ASC) (Rousseeuw 1987) combines the concepts of cluster cohesion and separation; it relates distances between objects within the same cluster with distances between objects in different clusters. The silhouette coefficient (s) of an object is calculated as:

$s = (b - a)/\max(b,a)$, where a is the average distance of an object to all the objects in the same cluster and b is the minimum average distance between an object to objects in any of the other clusters.

The average silhouette coefficient for each cluster is calculated by averaging the silhouette coefficients of all the objects in the cluster. An overall measure of the quality of the clustering is obtained by computing the average silhouette coefficient over of all objects in the data. Two other criteria (C-Index (Hubert, 1976) and method based on F_{ST}) for determining the optimum number of clusters are discussed in Appendices 2. In applying the criteria for determining optimum numbers of clusters, each dendrogram was cut into a specified number of clusters K(= 2, 3 … 10) and values of the criteria for determining the number of clusters were calculated and plotted against K. For both PBC and ASC, the number of clusters (K) at which the plot of K versus the value of the criterion is maximum is considered as the optimum number of cluster for a given data set. It should be noted that all these criteria do not directly test for the presence of one cluster (K =1).

2.2.5 Data analysis

Real data. After performing cluster analysis using UPGMA and Ward, CPCC and AC were calculated. The results from hierarchical cluster analysis were also compared with the results from STRUCTURE with regard to cluster composition and appropriate number of clusters.

STRUCTURE was run under the assumption of an admixture model with independent allele frequency model. No *prior* information was used. Calculations were carried with the number of subgroups K ranging from two to 10 with three independent repeats for each K and with 100,000 iterations of which the first 30,000 were used as burn-in.

Simulated data In this paper the analysis of variance (ANOVA) approach (algorithm described by (Yang 1998)) and implemented in Hierfstat package in R by (Goudet 2005) was used to calculate subgroup differentiation (F_{ST}). To explore the relationships between F_{ST} and clustering evaluation criteria, datasets from different simulations were pooled together and then grouped based on the strength of subgroup differentiation into groups (each containing 100 datasets) with similar realized values of F_{ST}. Hierarchical cluster analysis was performed using Agglomerative Nesting (Agnes) procedure (Kaufman and Rousseeuw 1990) of the package Cluster of R.

The ability of UPGMA and Ward to recover the subpopulations in the simulated data was evaluated using overall cluster purity (Zhao and Karypis 2004). Overall purity was calculated as follows. Let $p_{ij} = \frac{m_{ij}}{m_i}$ be the probability that a member of cluster i (i = 1,2,…, I) belongs in reality to subpopulation j (j = 1, 2,…., J), m_{ij} is the number of members of subpopulation j allocated to cluster i and m_i is the number of members of cluster i. The purity for each cluster (p_i) is defined as the maximum probability of correct assignment of cluster i to one of the subpopulations, i.e. $p_i = \max_j(p_{ij})$, and over all purity is defined as $\sum_{i=1}^{k} \frac{m_i}{m} p_i$.

2.3 Results

2.3.1 Coconut

Both dendrograms (UPGMA and Ward) resulted into two major clusters (Fig 1), but clear differences were evident within these clusters. For example, any attempt to produce more than two clusters from each dendogram result into groups of very different structures with UPGMA resulting into highly unbalanced clusters in terms of sizes, (many of the clusters contained one or two accessions) compared to Ward. UPGMA (CPCC = 0.82) preserved the original distance matrix better than Ward (CPCC = 0.74). The two dendrograms had very different values of AC (Ward: 0.97; UPGMA: 0.58).

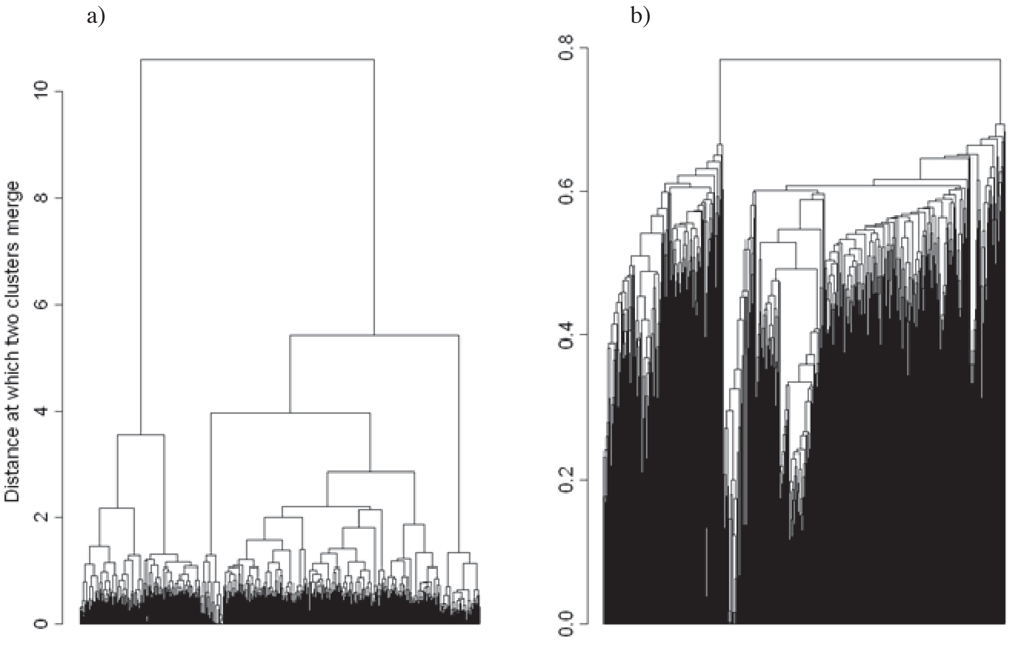

Fig 1: Dendrograms for the coconut data a) Ward; b) UPGMA. Dendrograms produced by Ward and UPGMA are clearly different with respect to branching. Ward dendrogram had Cophenetic Correlation Coefficient (CPCC) of 0.74 and Agglomerative Coefficient (AC) of 0.97 while UPGMA had CPCC of 0.82 and AC of 0.58. The two major clusters in the two dendrograms had similar compositions (Accessions associated with Indian and Atlantic Oceans versus those associated with the Pacific Ocean)

When applied to the Ward dendogram, both criteria for determining the optimum number of clusters (PBC and ASC) identified two as the optimal number of clusters for the coconut data (Fig 2 a) and b)). However, when applied to UPGMA dendrogram, PBC was not able to identify an optimum number of clusters *i.e*. changing the number clusters from two to ten produced very similar correlations (Fig 2 a). STRUCTURE (method by Evanno et al. 2005) also showed two as the optimum number of clusters (see Appendix 1).

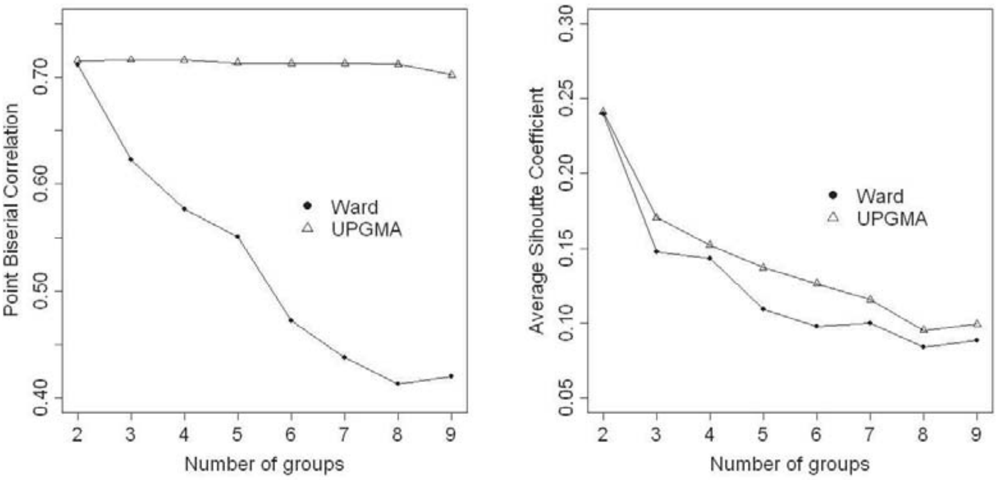

Fig 2: a) Plot of the Point-Biserial Correlation (PBC) versus the number of groups for the UPGMA and Ward dendograms for the coconut data. b) Plot of the Average Silhouette Coefficient (ASC) versus the number of groups for the UPGMA and Ward dendograms for the coconut data. For both criteria, the number of groups (K) for which the criterion is maximum (or point where the plot flattens off) indicates the optimum number of clusters. Both criteria show two as the optimum number of clusters

Composition of clusters

The two major groups identified by both UPGMA and Ward contained accessions associated with the Pacific Ocean versus accessions associated with the Atlantic and Indian oceans. These two major groups were also observed when clustering was done using STRUCTURE (K=2) (see Fig 3). While further subdivision obtained from Ward's dendogram led to formation of clusters/groups which coincided with groups based on passport data (region of origin), this was not possible with UPGMA. In terms of grouping of accessions, the results from STRUCTURE are quite similar to those of Ward. In fact, for the number of groups (K) equal two, three or four, the groups formed by STRUCTURE were almost identical to those produced by cutting Ward's tree to produce the same number of clusters (Fig 3). For example, by specifying (K = 3), both STRUCTURE and Ward resulted into the following three groups: 1) accessions associated with the Atlantic and Indian oceans 2) accessions from Central America (Panama) and 3) other accessions associated with the Pacific ocean. Similarity between groups formed by STRUCTURE and Ward was also observed for the potato data (see Appendix 1).

2.3.2 Simulated data

The two migration models (Island and Stepping stone) yielded identical results so only the results of the Island model will be shown. The simulated data sets varied greatly with respect to subpopulation differentiation with realized F_{ST} ranging from 0.010 to 0.431. In general, the values of CPCC increased with subgroup differentiation (expressed as F_{ST}); UPGMA produced a consistently higher CPCC than Ward (Fig. 4). The difference in CPCC between UPGMA and Ward decreased with increasing subgroup differentiation. AC also increased with subpopulation differentiation for both UPGMA and Ward (Fig. 4). In this case Ward showed a higher AC than UPGMA; Ward reached the maximum value of one with F_{ST} just over 0.1, *i.e*. the curve flattens off much quickly.

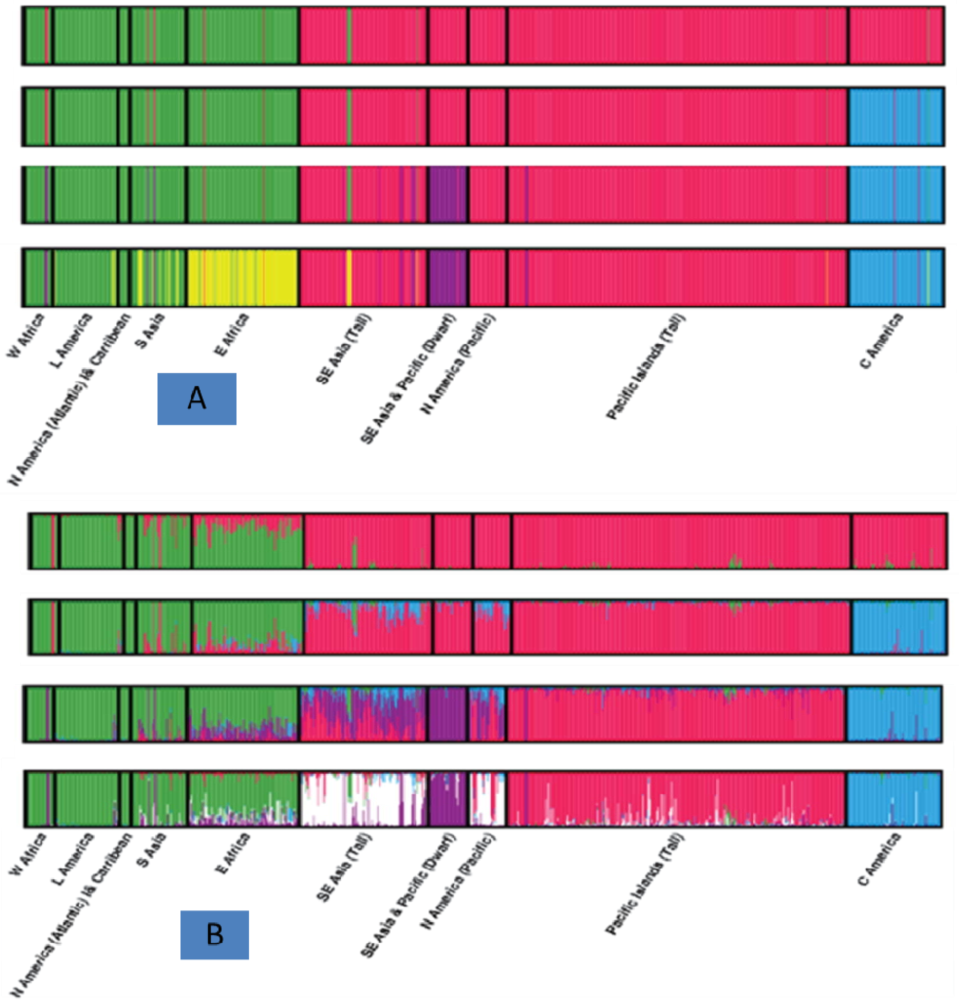

Fig 3: A) Bar plots for individual coconut accessions generated by cutting the Ward dendogram into a specified number of clusters/groups; the numbers of clusters from top to bottom were 2, 3, 4 and 5. The clusters are represented by different colours. Each column represents one accession. The labels below the bar plots indicate the regions of origin of the coconut accessions. **B)** Bar plots for individual coconut accessions generated by STRUCTURE 2.2 using the admixture model with independent allele frequency model based on 30 SSR markers; the numbers of clusters from top to bottom were 2, 3, 4 and 5. The groups are represented by different colours. Each bar is partitioned into segments indicating its genetic composition, the longer the segment the more an accession resembles one of the groups. The labels below the bar plots indicate the regions of origin of accessions.

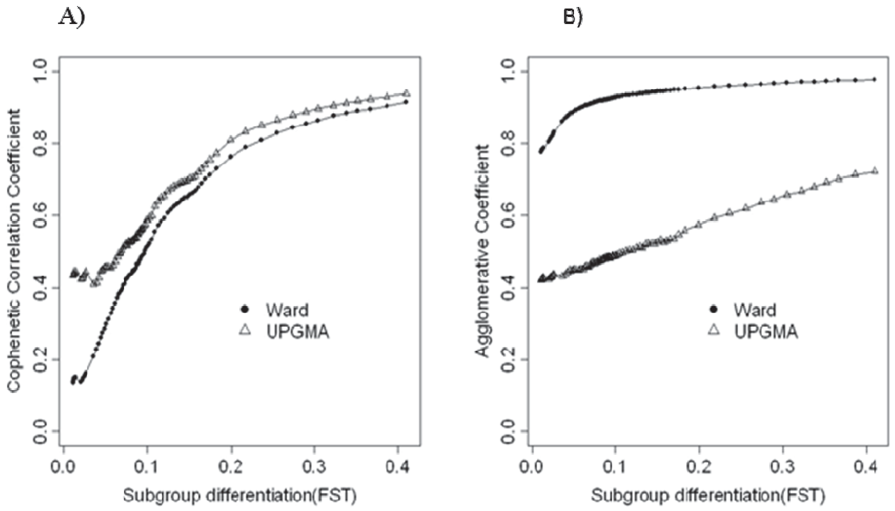

Fig 4: A) Relationship between Cophenetic Correlation Coefficient (CPCC) and subgroup differentiation (F_{ST}) for the simulated data A) Relationship between Agglomerative Coefficient (AC) and subgroup differentiation (F_{ST}) for the simulated data. Each data point is the average of 100 datasets with similar subgroup differentiation.

Identification of the optimum number of groups

Cutting of UPGMA trees resulted into highly unbalanced clusters (one or two clusters containing the majority of accessions with several other clusters with 1 or 2 accessions like in real data); only results for Ward is presented. The performance of the criteria for determining optimum number of clusters also depended on the amount of subgroup differentiation (Fig. 5). With relatively weak population differentiations (F_{ST} <0.08), all methods performed quite poorly in identifying the correct number of groups. At low differentiation levels, most criteria for determining optimum number of clusters gave two as the appropriate number of clusters. We also noticed that for a number of data sets with weak subgroup differentiations the values of criteria for determining optimum number of clusters either kept rising or falling, or kept fluctuating to an extent which did not allow determination of an optimum number of clusters. At higher levels of population differentiation (F_{ST} > 0.2) the performances of became similar.

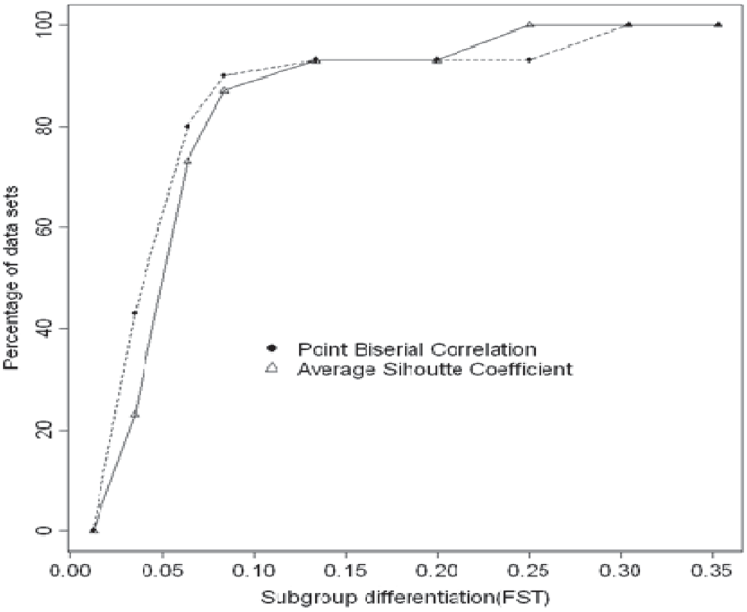

Fig 5: Percentages of simulated data sets for which the Point Biserial Correlation (PBC) and the Average Silhouette Coefficient (AC) identified the correct number of clusters versus the subgroup differentation (F_{ST}) (results from Ward only). Each point is based on 30 simulated data sets.

From Fig 6 it can be observed that Ward performed well in recovering the subpopulations. Except for relatively weak subpopulation differentiation ($F_{ST} < 0.05$), by cutting the trees into five groups, Ward produced clusters of which over 90% of the members were from one subpopulation. The poor performance of UPGMA methods in recovering the original subpopulations, even with high subgroup differentiation, is due to the fact that UPGMA produced highly unbalanced clusters.

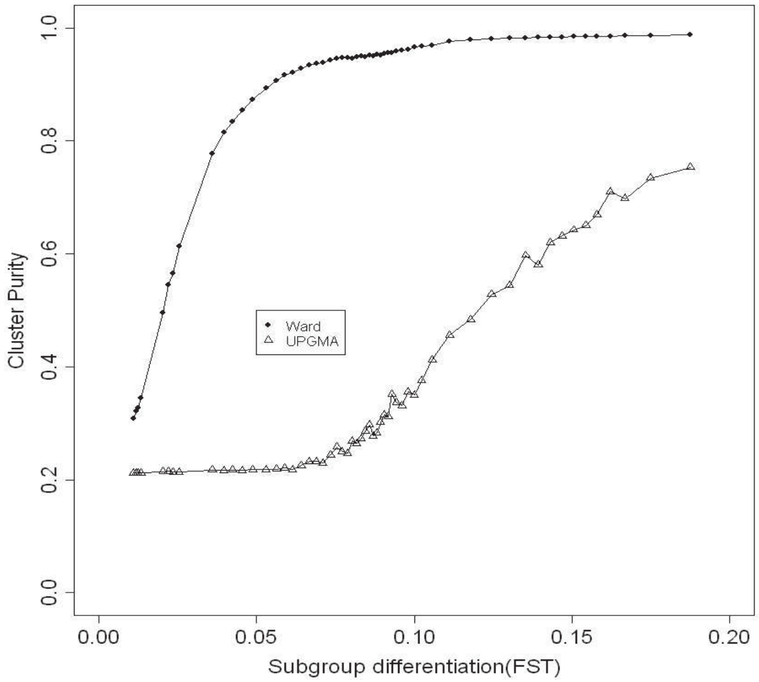

Fig 6: Plot showing the difference in ability of Ward and UPGMA to recover known subgroups in the data based on cluster purity. Each point is based on 100 datasets of similar F_{ST} values. Data sets with zero migration rates were excluded since we were mainly interested in low to medium subgroup differentiation.

2.4 Discussion

This paper shows that, if used with care, traditional cluster analysis provides a simple and powerful tool for determining the genetic structure of germplasm collections using molecular marker data. Traditional cluster analysis is available in many standard statistical packages and does not require special purpose software like STRUCTURE. In addition, when clustering individual accessions, the performance of hierarchical clustering techniques depends only on subgroup differentiation, not on the migration models used to simulate the data, provided that descrete subgroups are present.

Based on our results, CPCC can be used as an indicator for the strength of subgroup differentiation. A high CPCC ($CPCC \geq 0.8$) with both UPGMA and Ward is an indication of the presence of reliable population structure in the data. Although it has been shown theoretically and empirically that UPGMA always produce dendograms with a higher CPCC than other clustering algorithms (Farris 1969), our simulation results showed that, if distinct groups exist, the difference in CPCC between UPGMA and Ward is expected to be small and this difference gets smaller as subgroup differentiation increases. The differences in CPCC between Ward and UPGMA in real data also appear to reflect the degree of distinction between the groups in the data. For example, the common bean data with two distinct groups (Mesoamerican versus Andean) had a much smaller difference (0.07) in CPCC between Ward and UPGMA compared to potato data (0.17) with many unique accessions. For taxonomic applications (see Rohlf (1992)), it is recommended that CPCC should be very high ($CPCC > 0.9$) for a dendogram to be useful. Our results indicate that when clustering large numbers of accessions the CPCC obtained using Ward is not likely to be greater than 0.85 unless the subpopulations are highly differentiated (F_{ST} >0.25). This is due to the fact that Ward tends to form balanced clusters which may include outlying accessions (Jobson 1992); UPGMA tends to form unbalanced clusters assigning outlying accessions to separate clusters.

The usefulness of AC as a method for quantifying the amount of hierarchical structure in the data appears to be quite limited especially when applied to Ward. For Ward, the distance at which all clusters finally join is often much larger than the distance at which objects are joined in a cluster for the first time. All the three real data sets show very similar AC (0.97, 0.94, and 0.90 for coconut, potato and common beans respectively) with Ward but marked differences observed for UPGMA (0.58, 0.77, and 0.67 for coconut, potato and common beans respectively). Several studies in the literature have also obtained high AC values (≥ 0.95) with Ward and have used these results to either justify the use of Ward clustering algorithms or to conclude that there is substantial amount of structure in the data (Fan et al. 2004, Cushman et al. 2010, Negro et al. 2010). Based on our results which showed that Ward can result in a high AC even for a homogenuous population, these conclusions can be misleading. We suggest that further modification should be made before AC can be used in conjunction with Ward. It should be noted that AC was initially proposed to describe the strength of the hierarchical structure as obtained by UPGMA (Kaufman and Rousseeuw 1990). The rather low values of AC (<0.75) obtained from UPGMA dendograms even for highly differentiated subgroups could be attributed to a chaining effect (tendency of a clustering algorithm to pick out long string-like clusters (see Johnson and Wichern (2002)) caused by outliers. UPGMA dendrograms with high CPCC but a very low AC value (<0.6) often indicate the presence of many unique accessions or small groups of accessions (together with two or more large groups). The use of CPCC and AC (only with UPGMA) together can roughly tell us the degree of fit, the presence and strength of subgroup differentiation.

The poor performance of criteria for determining the number of clusters may be explained by the presence of weak subgroup differentiation found in many germplasm collections. Accessions in genebanks are not random samples but selections based on factors such as geographical distribution/location, accessibility or even perceived uniqueness. The inability of criteria to determine the optimum number of groups or clusters in a dataset is not limited to hierarchical cluster analysis techniques. Falush et al. (2003, 2007) stated that the method for determining the number of populations in STRUCTURE most often fails in real-world data sets due to various reasons (*e.g.* isolation by distance or inbreeding). The tendency for these criteria to show two as an optimal number clusters for the real data could be attributed to the presence of dominant groups (Evanno et al. 2005; Yan and Ye 2007). In the cases where dominant groups overshadow minor subdivision, sequential detection of structure as described by Yan and Ye (2007) could offer solutions. Based on the poor performance of criteria for determining optimum number of clusters with UPGMA, it is clear that when the cluster sizes are highly unequal, as will often be the case in germplasm collections, applying criteria for determining optimum number of clusters makes little sense. In the case of association studies, one way of getting around the problem of identifying optimal number of clusters could be to use the relatedness based on co-phenetic distances (predicted pair wise distances between accessions) directly to correct for population structure just like kinship or other relatedness information is used (K matrix). Studies have shown that correcting for population structure using the K matrix may be sufficient (see Zhao et al. 2007, Stich et al. 2008, Astle and Balding 2009). Our analysis show a high correlation between co-phenetic distances and dissimilarity between accessions based on the first two axes of principal coordinate analysis (see Appendix 2). However, further study is required to assess the usefulness of co-phenetic distance in association mapping studies.

Our simulation results showed that Ward was very successful in recovering the original subgroups in the data if they were present and distinctly separated. In addition, because the nature of groups formed by Ward, the dendrograms can be evaluated using standard criteria such as those for determining the number of clusters. However, in the presence of many unique or intermediate accessions the groups formed by Ward will not be homogeneous. In this case, the differences in CPCC between UPGMA and Ward can be quite helpful in deciding which method to select. In situations in which both UPGMA and Ward have high CPCC (≥ 0.8), Ward will have many advantages over UPGMA. However, in a situation in which only UPGMA has CPCC ≥ 0.8 and there is a big difference (>0.1) in the values of CPCC between UPGMA and Ward, it will be preferable to use the groups formed by UPGMA.

In conclusion, traditional cluster analysis (UPGMA and Ward) provides an easy and effective way for determining structure in germplasm collections. In addition to being simple to apply (using standard statistical software) and simple to interpret, it is possible to determine the presence and strength of subgroup differentiation as well as the presence and influence of unique accessions in the collection. It provides a good alternative for STRUCTURE or PCA in association analyses. It can be combined easily with mixed model facilities that are available in standard statistical packages. Although our simulations were based on random mating, similarity of results between the real data from both out-crossing (coconut and potato) and selfing species (common bean) clearly indicate that traditional cluster analysis can be applied in both mating systems.

Appendices

Appendix 1: Results of additional real data

a) Coconut

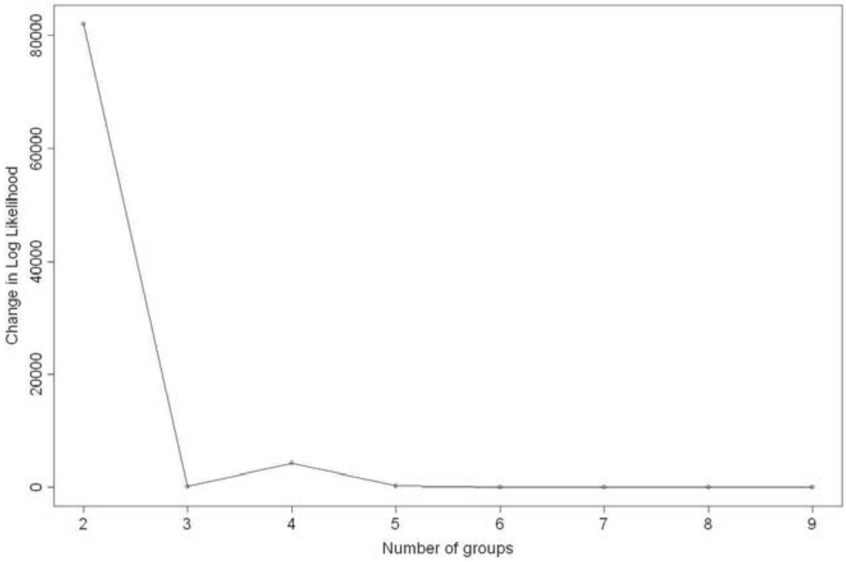

Fig 7: Detection of true number of groups (K) in the coconut data using method described by Evanno et al 2005. The programme was run for K=1 to 10 and for each K value, STRUCTURE was run 20 times. With this method, it is only possible to test for presence of more than one (K>1).

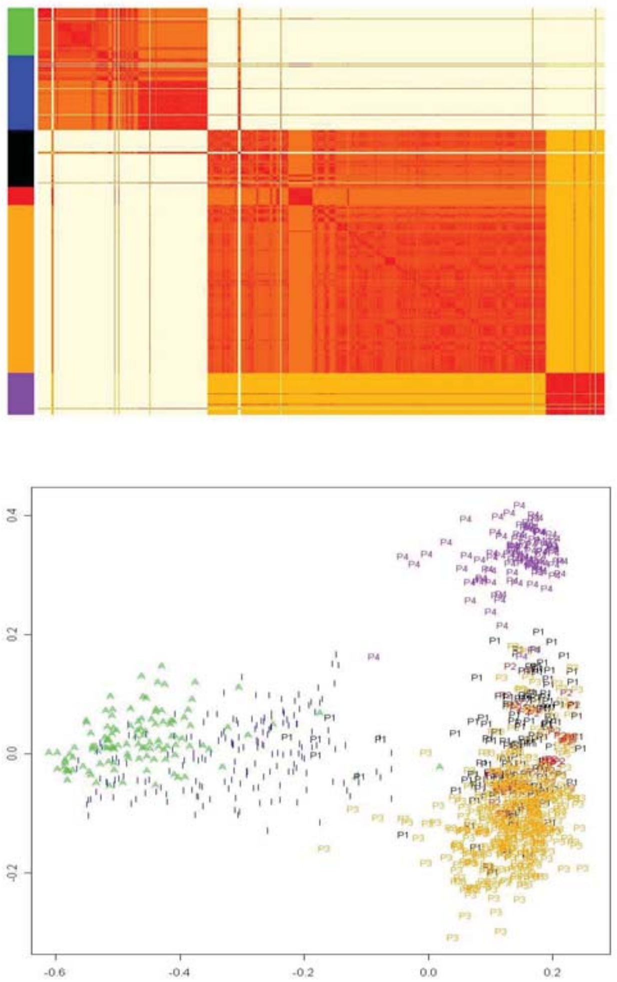

Fig 8 a)

Fig 8 b)

Fig 8: a) Heatmap of the relationships between accessions based on co-phenetic distances calculated using the Ward dendogram. The colours associated with the rows of the heatmap indicate the different regions from which the accessions were obtained b) Plot of the first two axes of a principal coordinate analysis with the letters and colours showing the regions from which the accessions were obtained (A (green)-Atlantic Ocean; I (blue)-Indian Ocean; P1 (black): Pacific Ocean (South East Asia); P2 (Red)-Pacific Ocean (dwarf); P3-Pacific Ocean (the Pacific Islands); P4-Pacific Ocean (Panama)).

b) Potato

Data The data used in this study consisted of 233 diploid accessions genotyped with 50 SSR markers. The accessions were collected from different regions of South America (Bolivia – 44; Colombia – 80; Ecuador – 16 and Peru - 91). Potato is an out-crossing species with a substantial level of self-pollination. The 233 diploid accessions came from four species (*S. ajanhuri* (22); *S. goniocalix* (47); *S. phureja* (105) and *S. stenotomum* (59)).

Dendrogram, CPCC and AC Dendrograms are given in Fig 9. The potato data showed many more differences between the results of the different clustering algorithms than the coconut data. Ward (CPCC = 0.62) performed poorly in preserving the original pair wise distances between accessions compared to UPGMA (CPCC = 0.89). With regard to quantification of the hierarchical structure the difference between Ward (AC = 0.94) and UPGMA (AC = 0.77) was smaller than for the coconut data (0.97 for Ward versus 0.58 for UPGMA).

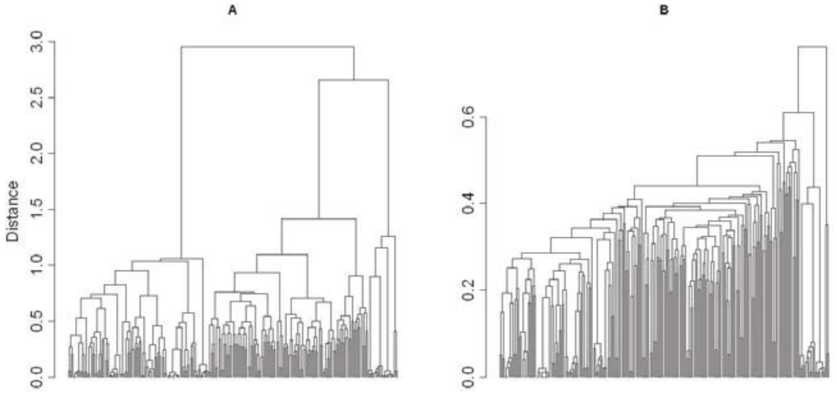

Fig. 9: Dendrograms for potato for Ward (A) and UPGMA (B). Clear differences can be observed amongst the clustering techniques**.** Ward dendrogram had Cophenetic Correlation Coefficient (CPCC) of 0.62 and Agglomerative Coefficient (AC) of 0.94 while UPGMA had CPCC of 0.89 and AC of 0.77.

Determining the optimum number of clusters The criteria for determining the number of clusters applied to the Ward did not agree on the optimum number of clusters (PBC: 4; C-index: 2; ASC: 6 and F_{ST}-based method: 3). C-index had local optima at four and three clusters (Fig. 10). A similar disagreement was observed with the UPGMA dendrogram (PBC: 3; C-Index, F_{ST} and ASC: 2). It should be noted that the groups resulting from the two dendrograms were of different sizes and compositions. For STRUCTURE, the plot of log-likelihood versus the number of groups K did not provide a clear indication of the optimum number of clusters. However, for potato it is clear that the number of clusters is less than eight (there is a sharp drop after k=5).

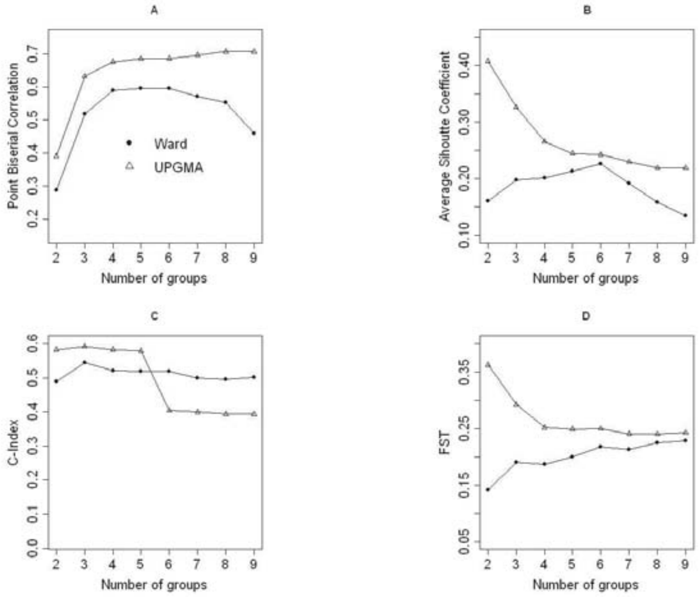

Fig 10: Plot of the criteria for determining the optimum numbers of clusters for UPGMA and Ward dendrograms for potato data. For PBC (A), ASC (B) and FST-based criteria (D), the number of clusters with the maximum value of the criteria (or the number where the graph starts leveling off) is the optimum; the opposite applies to C-index (C).

Composition of clusters While Ward split accessions into two major clusters *S. ajanhuri* (mainly accessions from Bolivia and Peru) versus the other species (*S. goniocalix*, *S. phureja* and *S. stenotomum*; accessions from Colombia and Ecuador), UPGMA first isolated three accessions of *S. ajanhuri* (all from Bolivia) from all other accessions. As for coconut, most clusters formed by cutting UPGMA trees consisted of 1 or 2 accessions.

In terms of composition of clusters, results of STRUCTURE and Ward showed a good agreement (see Fig. 11). For example, for K =2 STRUCTURE and Ward both split the accessions into *S. ajanhuri* (from Bolivia and Peru) versus *S. goniocalix*, *S. phureja* and *S. stenotomum* (from Colombia and Ecuador).

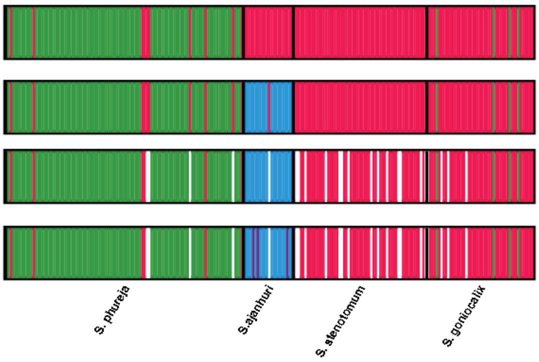

Fig 11 a) Bar plots for individual potato accessions generated by cutting the Ward dendrogram into 2, 3, 4 or 5 groups (from top to bottom). Groups are represented by different colours. Each column represents one accession. The labels below indicate the potato species.

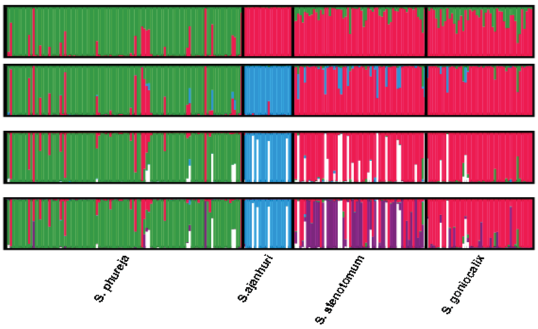

Fig 11 b) Bar plot for individual potato accessions generated by STRUCTURE 2.2 using the admixture model with independent allele frequencies based on 50 SSR markers for 2, 3, 4 or 5 groups (from top to bottom). Groups are represented by different colours. Each column represents one accession. Bars may consist of different segments representing its composition; the longer a segment the more an accession resembles the corresponding cluster. The labels below indicate the potato species.

c) Common Bean (*Phaseolus vulgaris*)

Data The data consisted of 603 accessions with 296 being described as Andean and 307 as Mesoamerican types genotyped with 36 SSR markers. These accessions originated from 24 different countries, most of them coming from Peru (184), Mexico (183), Guatemala (62), Ecuador (37), Colombia (30) and Brazil (24).

Dendrogram, CPCC and AC Dendrograms are given in Fig. 12. For common bean, both clustering methods preserved the original pair wise distances between the accessions quite well. With a CPCC of 0.92, UPGMA performed better than Ward (0.85). Ward indicated the presence of hierarchical structure better than UPGMA (AC of 0.97 versus 0.66).

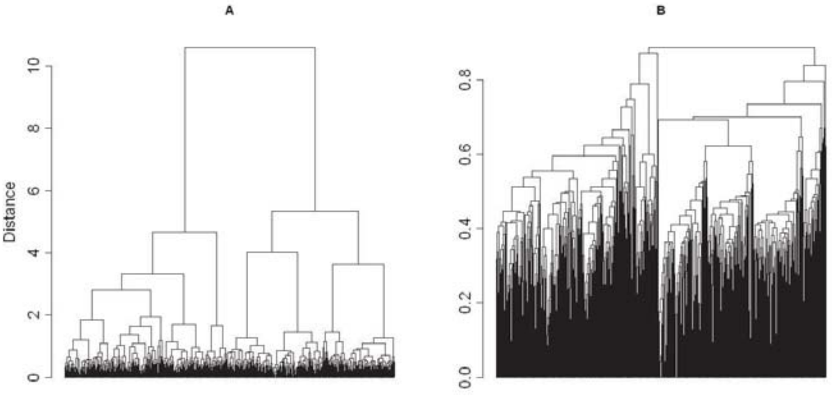

Fig 12 Dendrograms for common bean for Ward (A) and UPGMA (B); dendrograms are clearly different with respect to branching. Ward dendrogram had Cophenetic Correlation Coefficient (CPCC) of 0.85 and Agglomerative Coefficient (AC) of 0.97 while UPGMA had CPCC of 0.92 and AC of 0.66. The two major clusters in the two dendrograms had similar compositions (Andean versus Mesoamerican type)

Determining the optimum number of clusters The criteria for determining the optimum number of clusters produced conflicting results for the common beans(Fig. 13) and in most cases it is not straight forward which ***k*** produce optimum value of the criteria. For Ward, the following approximate optima were found, PBC: 4, ASC 2 and F_{ST}: 6. For the C-index it was not possible to determine an optimum number of clusters. For UPGMA, the optimum number number of clusters were PBC: 6, ASC: 2, FST: 6. Also, for UPGMA C-index did not indicate an optimum number of clusters.

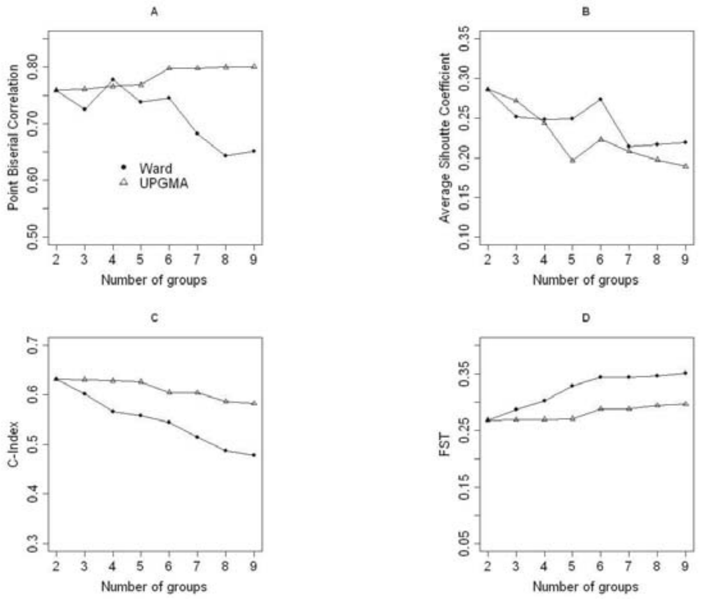

Fig: 13: Plot of the values of criteria for determination of optimal number of clusters against the number of clusters for both UPGMA and Ward dendrograms. For PBC (A), ASC (B) and FST-based criteria (D), the number of clusters with the maximum value (or where the graph starts leveling off) of the criteria is the optimal number of clusters; the opposite applies to C-index (C).

Composition of clusters Cutting the UPGMA and Ward dendrograms into two groups led to the separation of the Andean and Mesoamerican types. Further cutting of the UPGMA dendrogram resulted into highly unbalanced clusters with respect to size. For example, with six clusters, three clusters contained three or fewer accessions.

Appendix 2: Additional results from simulated data

Fig. 14 shows a sample of dendrograms for Ward and UPGMA obtained using simulated data sets. These dendrograms show again that usually Ward dendrograms are highly balanced, dividing objects in major groups, whereas UPGMA dendrograms are usually highly unbalanced, forming small groups of objects.

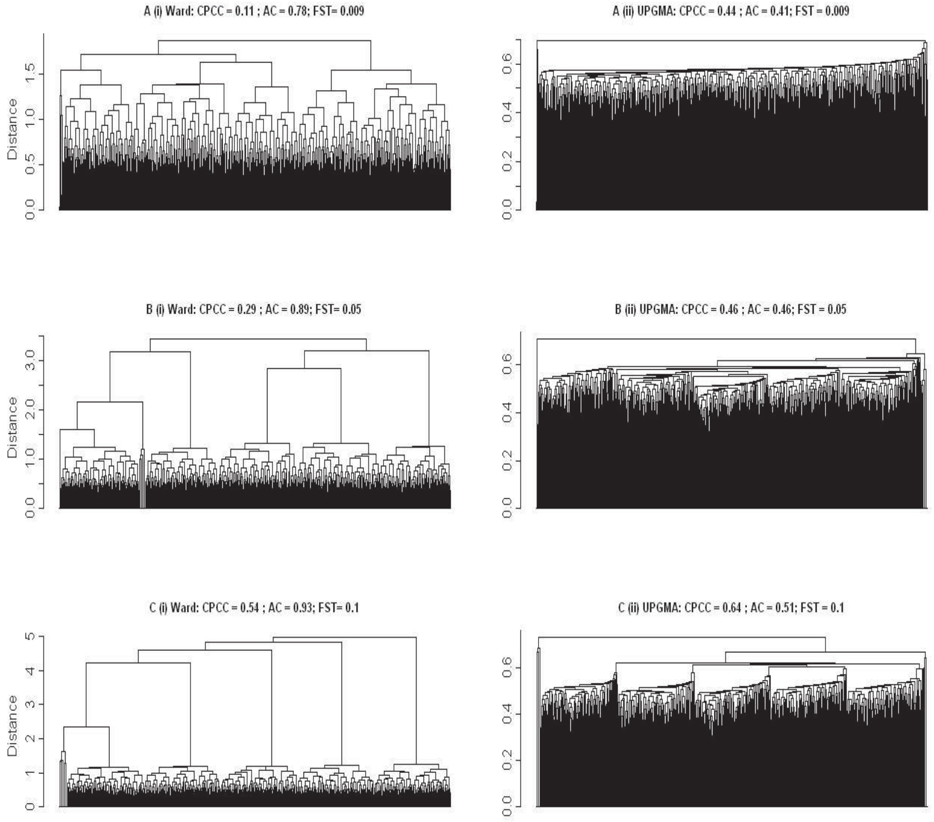

Fig 14: UPGMA and Ward dendrograms for three simulated data sets of different subpopulation differentiations (F_{ST} = 0.009 (A), 0.05(B) and 0.1(C)). The dendrograms show changes in CPCC, AC and branching patterns as subgroup differentiation increase from A to C.

Determining the optimum number of clusters From the simulations, it was only possible to get sensible results when the criteria for determination of optimum number of clusters were applied to Ward. Cutting of UPGMA dendrograms resulted into highly unbalanced groups. The performance of the criteria for determining the optimum number of clusters rules also depended on the level of differentiation between subpopulations(see Table 2). The simulation results indicated that with weak population differentiation (F_{ST} <0.08), all methods performed quite poorly in identifying the correct number of groups. With relatively weak differentiation between subpopulations, most criteria for determination of optimum number of clusters indicated two as the appropriate number of clusters. We also noticed that with weak differentiation between subgroups values of the criteria kept fluctuating to the extent that it was not possible to determine a knee or a dip indicating an optimal number of clusters. Beyond a certain level of population differentiation (F_{ST} > 0.2) the performance of all criteria become quite similar (see Table 2).

Table 2 Percentage of simulated data sets (based on 30 datasets per group) in each category for which each criteria for determining the number of clusters identified the correct number of clusters (results from Ward only)

	Criteria			
Group mean F_{ST}	**ASC (%)**	**PBC (%)**	**C-index (%)***	**F_{ST} (%)****
0.0123	0	0	3.3	20
0.0347	23	43	27	20
0.0637	73	80	50	77
0.0836	87	90	53	97
0.1335	93	93	67	**100**
0.1998	93	93	77	**100**
0.2503	**100**	93	**100**	**100**
0.3039	**100**	**100**	**100**	**100**
0.3528	**100**	**100**	**100**	**100**

**C-Index*: This criterion is only based on distances between objects within clusters and is calculated as follows: $C-Index = (S - S_{\min})/(S_{\max} - S_{\min})$

in which *S* is the sum of pair wise distances between objects within the same cluster summed over all clusters. If l is the number of pairs of objects used to calculate *S*, then $S_{\min}$ and $S_{\max}$ are the sum of the *l* smallest and the *l* largest distances between all pairs of objects (*i.e.* ignoring the presence of clusters).

***F_{ST}-based criterion:* F_{ST} directly measures genetic divergence among clusters. Wright (1951; 1965) defined F_{ST} as the correlation between two alleles chosen at random within a subpopulation relative to alleles sampled at random from the total population. In this case, F_{ST} is calculated between clusters obtained by cutting dendrograms into specified numbers of clusters. Theoretically, the optimum number of clusters should result in the highest F_{ST}-value. In this paper the analysis of variance (ANOVA) approach was used to calculate F_{ST}, more specifically the algorithm of Yang (1998) as implemented in the Hierfstat package of R (Goudet 2005).

Chapter 3

Principal components analysis improves ability of hierarchical cluster analysis methods for recovering the genetic structure of germplasm collections

Abstract

Understanding the genetic structure of germplasm collections is a prerequisite for effective and efficient utilization of genetic resources stored in genebanks. Although recent developments in genetics and statistics have led to the development of new tools for studying genetic structure of populations, the old, and usually simpler approaches such as hierarchical cluster analysis are still very popular with scientists. Our study explores the potential of combining two classical multivariate statistical techniques, cluster analysis and principal component analysis (PCA), for understanding the genetic structure of germplasm collections. The two-step approach involves first applying PCA to molecular marker data followed by cluster analysis using significant principal components (PC) only. The determination of the number of significant PC is done using the Tracy-Widom (TW) distribution. The parameters of the TW distribution only depend on the dimensions of the allele frequency matrix. In this study we compared the performance of cluster analysis (Ward and model-based hierarchical clustering) using reduced sets of significant PC with cluster analysis using the full data set. For reduced sets of PC, Ward's clustering was performed on Euclidean distances, while for the full data sets three other distance measures (proportion of shared alleles, Jaccard and simple matching) were used. Clustering (Ward and model-based clustering) using reduced sets of PC performed much better than clustering using full data sets both in terms of recovering groups as well as in determining the exact number of groups. The improvement in performance was most noticeable in cases with low population structure. In conclusion, PCA in combination with cluster analysis provides a very useful tool for studying genetic structure of heterogeneous germplasm collections, which can be carried out using standard statistical software.

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3.1 Introduction

Knowledge of the genetic structure of heterogeneous germplasm collections is essential when forming core collections (Brown 1995; van Hintum et al. 2000), and in association studies (Wang et al. 2005; Shriner et al. 2007). Hierarchical clustering techniques such as Ward and UPGMA are still among the most-used methods for determining structure, and a recent study by Odong et al. (2011) indicates that they are also very useful when molecular markers have been used to characterise the collection. Unlike programs such as STRUCTURE (Pritchard et al. 2001), hierarchical clustering techniques require little computer time, and moreover, they are simple to use. However, both traditional clustering algorithms and programs such as STRUCTURE do not always perform very well with germplasm data especially when it comes to the determination of the number of clusters. Principal component analysis (PCA) has been suggested to enhance the performance of clustering techniques (Patterson et al. 2006, Lee et al. 2009). In this study, we explore the possibility of boosting the performance of hierarchical cluster analysis using PCA.

Recent developments in population genetics theory have provided interesting avenues for exploiting the information that molecular markers contain about population differentiation. It has been shown that there is a direct theoretical relationship between population genetic structure and principal components (Patterson et al. 2006, McVean, 2009). In particular, the distribution of eigenvalues associated with principal components is determined by the number of independent sources of differentiation (i.e. subpopulations) present in the dataset. Moreover, the distance between groups along the major PCs has been shown to be proportional to the level of genetic differentiation (McVean, 2009). PCA has been successfully used with SNP data for determining the number of different populations (Patterson et al. 2006) and to assign individuals to these populations (Lee et al. 2009). The usefulness of this novel application of PCA in understanding the genetic structure of germplasm collections is yet to be exploited, especially using multi-allelic markers such as Single Sequence Repeat (SSR) markers. SSR markers are still among the most commonly used molecular markers for germplasm characterization. For PCA to be useful for determining the number of subpopulations, the assumptions are a) the number of markers is greater than the number of individuals and b) the molecular markers used should be independent or unlinked (Patterson et al. 2006). It should be noted that unlike the SNP data used in previous studies (Patterson et al. 2006; Lee et al. 2009) in which the markers are usually much greater in number than the individuals, in most germplasm collection data, this difference (between number of individuals and number of markers) is much smaller. In addition, when SSR markers are treated as binary markers (each allele is coded 0 (absent) or 1 (present)), the assumptions of independence of the different columns in the data matrix is violated.

Because of the multi-allelic nature of SSR markers, various methods for determining the (dis)similarity (hereafter referred to as distances) between individuals or subgroups exist; there is no standard way of handling SSR markers. For example, when SSR markers are treated as binary markers (each allele is coded for presence or absence), a binary-based distance measure such as Jaccard (Jaccard 1908) can be applied (Anthony et al. 2002; Cordeiro et al. 2003; Balestre et al. 2008). Another common distance measure for SSR markers is based on the proportion of shared alleles (Chakraborty and Jin 1994; Chang et al. 2009). The performance of different distance measures for cluster analysis has not yet been extensively evaluated. In this study, we use real and simulated data sets to explore the effect of data reduction using PCA on the clustering of germplasm collections using a traditional hierarchical clustering technique (Ward's method) and a model-based hierarchical clustering technique (Mclust, mixture of normal distributions; Fraley and Raftery 2002; Fraley 2006). Ward and UPGMA are the two most commonly used hierarchical clustering techniques in plant germplasm studies. Ward was selected for this study because it has been shown to perform much better with molecular marker data than UPGMA (Odong et al. 2011). Model-based clustering was used previously with SNP data and shown to perform quite well (Lee et al. 2009). We also evaluated the effect of different distances measures (Euclidean, proportion of shared alleles (Bowcock et al. 1994, Chang et al. 2009), Jaccard (Jaccard 1908) and simple matching (Sokal and Michener 1958) for clustering germplasm collections using SSR markers.

3.2 Materials and Methods

3.2.1 Description of data sets

Real data

A coconut (*Cocos nucifera*) data consist of 1014 accessions genotyped with 30 SSR markers. The accessions were collected from different regions of the world (West Africa – 32; North America – 52; South Asia – 62; Latin America – 72; Central America & the Caribbean – 109; East Africa – 124; South East Asia – 183; the Pacific Islands - 380). Coconut is a diploid, mainly out-crossing species.   Most of the accessions in this collection are described as tall; only 43 dwarf accessions mainly from South East Asia were present. Dwarf coconuts have a high degree of self-fertilization. More than half (19) of the 30 SSR markers have known positions on the linkage map; they are well spread across the genome.

Simulated data

Marker data were simulated using SimuPOP (Peng and Kimmel 2005), a forward-time population genetic simulation environment. We used a finite island model (Wright 1931) and a stepping stone (Kimura 1953) migration model. In each generation, random mating (with 2% selfing) was assumed to produce diploid genotypes for 30 unlinked loci. We simulated 1000 individuals in five and eight subpopulations and 750 individuals in 15 subpopulation with varying levels of subpopulation differentiation (differentiation between subpopulations is determined by migration rate and number of generations). The migration rates used in this study were 0, 1 and 2 migrants per subpopulation per generation . At each of the 30 loci, the average allele frequencies of the coconut data were used as the starting allele frequencies for the simulation. Within each parameter set, all loci had the same mutation dynamics, according to a K-allele model (KAM). Under the KAM model, there are K possible allelic states, and any allele has a constant probability of mutating into any of the other K–1 allelic states (Crow and Kimura 1970). A mutation rate of 2 x 10^{-5} with 50 possible allelic states was used in the simulation. The mutation parameters were set to mimic highly polymorphic markers such as SSR markers. However, in this case the role of mutation is very limited since we used a limited number of generations in the simulation. In addition to using alleles from real data as starting frequencies for simulation, the numbers of generations for the simulations were restricted (from 5 to 200 generations) to mimic the situation of agricultural crops in genebanks.

3.2.1 Genetic distance measures

Because SSR markers are multi-allelic in nature genetic similarities or dissimilarities (hereafter referred to as distances) between individuals or groups are calculated in several ways. In this paper, four different types of genetic distances between accessions were used.

a) Distance based on proportion of shared alleles

The genetic distance (*D*) between individuals (accessions) *i* and *j* based on the proportion of shared alleles (*PSA*) was calculated as

$$D\_PSA_{ij} = 1 - PSA_{ij},$$

Where $PSA_{ij} = \sum_{l=1}^{L}\sum_{a=1}^{A_l} \min(f_{ila}, f_{jla}) / L$.

In diploids f_{ila} and f_{jla} represent the frequencies of allele a (a =1, 2... A_l ; A_l is the total number of alleles for molecular marker l (l =1, 2... L) in individuals *i* and *j*, respectively; $i \neq j = 1, 2 \dots N$). It should be noted that in this paper PSA_{ij} refers to proportion of shared alleles between (diploid) individuals rather than populations; as a consequence f_{ila} , f_{jla} = 0, $\frac{1}{2}$ or 1. For more information on the proportion of shared alleles as similarity measure between populations, see Bowcock et al. (1994), Chakraborty and Jin (1994) and Chang et al. (2009).

b) Euclidean distance

The Euclidean or straight line distance between two individuals i and j, having observations on P quantitative variables denoted as $x_{i1}, x_{i2}, ..., x_{iP}$ and $x_{j1}, x_{j2}, ..., x_{jP}$ is given by

$$D\_Eucl_{ij} = \sqrt{(x_{i1} - x_{j1})^2 + (x_{i2} - x_{j2})^2 + ... + (x_{iP} - x_{jp})^2} \ .$$

In this study the variables $x_1, x_2, ..., x_P$ are the principal components obtained from the molecular marker data.

c) ***Distances based on coding SSR markers as binary data***

For the calculation of genetic distances between individuals, it is common practice to treat SSR markers as binary markers (Anthony et al. 2002; Cordeiro et al. 2003; Balestre et al. 2008). In this case, each allele is treated as a binary variable (0 = absence, 1=presence). Because they are among the most frequently used distances in the genetic diversity studies, the Jaccard (Jaccard 1908) and simple matching (Sokal and Michener 1958) distances have been selected for this study. The major difference between the two distances is that for Jaccard double-absent matches are ignored, while for simple matching they are included. It should be noted that the double-absent matches do not contain useful information in the case of multi-allelic SSR markers. However, simple matching distance is included in this study for reference purposes. For each allele, the results of individuals i and j can be summarized in a contingency table:

		Individual *i*	
		Present	Absent
Individual *j*	Present	a_{ij}	b_{ij}
	Absent	c_{ij}	d_{ij}

where $a_{ij} + b_{ij} + c_{ij} + d_{ij} = A$ (A is total number of alleles for all the L SSR markers). The distances based on binary coding of SSR markers are calculated as follows

i) ***Jaccard distance***

$$D\_JAC_{ij} = \sqrt{1 - \frac{a_{ij}}{a_{ij} + b_{ij} + c_{ij}}}$$

ii) ***Simple matching distance***

$$D\_SM_{ij} = \sqrt{1 - \frac{a_{ij} + d_{ij}}{a_{ij} + b_{ij} + c_{ij} + d_{ij}}}$$

It is common to convert similarity measures into distances without using the square root, but the square root gives distances the Euclidean property (Gower and Legendre, 1986). The Euclidean property is important, because it is a requirement of many multivariate analysis methods such as principal coordinate analysis, hierarchical cluster analysis, hierarchical classification, and graph theory (Gower, 1985). However, empirically we found that conversion of similarity measures into distance measures with or without using the square root had no effect on the formation of clusters.

3.2.3 Principal component analysis

PCA does not attempt to classify individuals into discrete subgroups but instead it characterizes each individual by coordinates (PC) along the major axes of variation. In this paper we investigate how these coordinates can be used to improve the results of both hierarchical and model-based clustering methods.

We treat each allele from an SSR marker l as a bi-allelic marker. The data can be represented as a rectangular matrix **G** of which the number of rows is equal to number of individual accessions N and the number of columns is equal to the total number of alleles (A) ($A = \sum_{l=1}^{L} A_l$, where A_l is the number of alleles from SSR marker l (l= 1, 2 …, L)).

The matrix **G** contains allele counts 0, 1 or 2. Before performing PCA, the matrix **G** is standardized by subtracting column means followed by dividing columns by their standard deviation (see Patterson et al. 2006). We performed PCA using the function *prcomp* in R.

Determination of eigenvalues and the Tracy Widom test

Eigenvalues

It has been shown recently that, in the absence of genetic structure and for independent markers, the leading eigenvalue of the covariance matrix of a normalized **G** matrix follows a Tracy-Widom (TW) distribution (Tracy 1994; Patterson et al. 2006). This fact has been exploited to determine the number of genetically different groups in genotypic datasets (Patterson et al. 2006). If there are k genetically different groups in a dataset, the number of significant eigenvalues based on TW distribution is expected to be k-1 (Patterson et al. 2006). In performing the TW test, Patterson et al. (2006) assumed that the SNP being analyzed were independent. It is clear that this assumption will be violated when the TW test is applied to SSR data since alleles from the same SSR marker are not independent. To get around this problem, we have adapted the procedure to handle SSR markers as follows:

a) Perform PCA on a matrix $\mathbf{G}_l$ (l =1,2,...L, L is the number of SSR), the sub-matrix of matrix **G** containing only alleles from SSR marker l. Let $\mathbf{G}_l$* (l=1, 2, ….., L) be the matrix of which the columns consist of PC (from matrix $\mathbf{G}_l$) explaining more than 0.5% of the variance. Removing PC which explain less than 0.5% of the variance eliminates noise so that it will become easier to detect the correct number of major groups. Removing non-important PC is similar to the idea of determination of the effective number of alleles.

b) Form a matrix $\mathbf{G}^*$ of which the columns consist of the columns of the matrices $\mathbf{G}_1$*, $\mathbf{G}_2$*,..., $\mathbf{G}_L$* obtained from step (a) above. The columns of the matrix $\mathbf{G}^*$ are thus effectively independent (when the loci (SSR markers) are independent) leading to an approximate TW distribution of eigenvalues (Tracy, 1994). The assumptions that markers are independent still holds.

c) Perform an eigenvalue decomposition on the matrix $\mathbf{X} = \frac{1}{n'}(\mathbf{G}^{**})(\mathbf{G}^{**})'$ where **G**** is obtained by standardizing the matrix **G*** (from each column of matrix **G***, we subtract the column mean and divide by its standard deviation) and n' is the number of columns of matrix **G*** .

Tracy-Widom distribution

Patterson et al. (2006) provided a detailed description of the TW distribution and its application for the detection of population structure. A brief review is provided below. Following the notation of Patterson et al. (2006), consider an $m \times n$ matrix **M** with ($m < n$),of which each entry contains an independent standard normal random variable. Let $\mathbf{X} = \frac{1}{n}\mathbf{MM}'$, and let $\{\lambda_k\}_{1<k<m}$ be the eigenvalues of **X**. For ordered eignenvalues ($\lambda_1 > \lambda_2 ... > \lambda_m$) Johnstone (2001) showed that for a suitably normalized matrix **M**, and for large m and n the largest eigenvalue λ_1 approximately follows a TW distribution (Tracy and Widom, 1994) with mean $\mu(m,n)$ and standard deviation $\sigma(m,n)$ in which

$$\mu(m,n) = \frac{(\sqrt{n-1} + \sqrt{m})^2}{n}$$

$$\sigma(m,n) = \frac{(\sqrt{n-1} + \sqrt{m})}{n}\left(\frac{1}{\sqrt{n-1}} + \frac{1}{\sqrt{m}}\right)^{1/3}$$

i.e. the statistic ($z = \frac{\lambda_1 - \mu(m,n)}{\sigma(m,n)}$) follows approximately a standard TW distribution. Patterson et al. (2006) state, that if the first k eigenvalues have been declared significant, the test for $\lambda_{k+1} > \lambda_{k+2} ... > \lambda_m$ can be carried out as **X** having dimension $(m-k)$ x $(m-k)$, and changing mean and variance of the TW distribution accordingly.

When analysing data from germplasm collections, the number of markers (*n*) is usually less than the number of individuals ($m > n$) so based on Johnstone (2001), we suggest that the above formulae must be adapted before performing the TW test (*i.e. m* = *min*(*nrow*(**M**), *ncol*(**M**)) and n = *max*(*nrow*(**M**), *ncol*(**M**)) where *nrow*(**M**) and *ncol*(**M**) are the number of rows and columns of the matrix **M** respectively).

Additional methods for determination of the number of groups

In addition to the TW test we also used two other criteria frequently used for determining the number of clusters in hierarchical cluster analysis: the point biserial correlation (PBC) and the average silhouette coefficient (ASC).

PBC (Milligan 1981) is defined as the correlation between the original distance matrix and a matrix consisting of zeros and ones indicating whether two objects are in the same cluster or not. This is an easy measure of the resemblance between the distance matrix (observed relationships) and the resulting tree (fitted relationships).

ASC (Rousseeuw 1987) combines the concepts of cluster cohesion and separation; it relates distances between objects within the same cluster with distances between objects in different clusters. The silhouette coefficient (s_i) of an object *i* is calculated as: $s_i = (d_{\mathrm{w(i)}} - d_{\mathrm{b(i)}})/\max(d_{\mathrm{w(i)}}, d_{\mathrm{b(i)}})$, where $d_{\mathrm{w(i)}}$ is the average distance of an object *i* to all the individuals in the same cluster and $d_{b(i)}$ is the minimum of the average distance between an object *i* and objects in another single cluster (i.e. for every cluster to which object *i* does not belong, the average distance between an object *i* and objects in that cluster is calculated (separately), and the minimum of those averages is $d_{b(i)}$). The average silhouette coefficient for a cluster is calculated by averaging the silhouette coefficients of all the objects in the cluster. The overall measure of the quality of the clustering (ASC) is obtained by computing the average silhouette coefficients over of all objects in the data.

3.2.4 Cluster analysis results

We explored the potential of using significant PC (expected to be the first *k*-1 PC in a situation with *k* subpopulations) to improve the performance of hierarchical clustering. Ward's clustering algorithm (Ward, 1963; Johnson and Wichern, 2002) was selected for this study because it is one of the most used clustering methods and has been shown to perform relatively well (see Odong et al 2011). Ward's method employs an analysis of variance (ANOVA) approach for calculating distances between clusters. For each pair of clusters, at each step in the clustering process, the effect of the union of every possible pair of clusters is considered, and the two clusters that produce the smallest increase in within group sum of squares are joined. Ward's method was used with a) Euclidean distances based on different numbers of PC b) distances based on the proportion of shared alleles c) distances based on SSR markers coded as binary markers (Jaccard and Simple matching).

The performance of cluster analysis using Ward's method was compared with that of model-based Gaussian hierarchical clustering (Fraley, 1998) using significant PCs. This method assigns individuals to groups by fitting a mixture of multivariate normal distributions to the data. The estimation of model parameters and assignment of accessions to groups is done by the Expectation Maximization (EM) algorithm (Banfield and Raftery, (1993)). In this case the geometric features (shape, volume and orientation) of the clusters are determined by the covariance structures. We used the implementation provided by the R package *mclust* (Fraley and Raftery, 2006). Several covariance models were tested and the spherical variable volume was found to fit our data best, and this model was used for all subsequent analyses. In this model, all clusters are assumed to have a spherical shape with different volumes depending on the variance within each cluster (see review by Fraley and Raftery, 2002).

Evaluating the performance of cluster analysis based on different genetic distances

In this study, the success of cluster analysis was measured using i) cluster purity and ii) the adjusted rand index (see below). The adjusted rand index was also used to compare the similarity of groups formed by clustering using different distance measures. To explore the effect of the number of PCs used in cluster analysis, we looked at the correlation between the group membership matrix and Euclidean distances between accessions based on the first *n* PC (only for simulated data). The performances of the two criteria for evaluation of the number of clusters were compared with testing for the number of subgroups using the TW distribution.

Cluster purity

For the simulated data, the ability of clustering techniques to recover the subpopulations was evaluated using overall cluster purity (Zhao and Karypis 2004). Overall cluster purity is calculated as follows. Let $p_{rq} = \frac{w_{rq}}{w_r}$ be the probability that an accession is allocated to cluster r (r = 1,2, …, R) belongs in reality to subpopulation q (q = 1, 2,…., Q), w_{rq} is the number of members of subpopulation q allocated to cluster r and w_r is the number of objects in cluster r. The purity for each cluster (p_r) is defined as the maximum probability of correct assignment of objects in cluster r to one of the subpopulations, i.e. $p_r = \max_q(p_{rq})$, and over all purity is defined as $\sum_{r=1}^{R} \frac{w_r}{w} p_r$. For the coconut data, the groups formed by clustering using different genetic distances were related to their passport data (country of origin).

Adjusted rand index

The adjusted rand index (Hubert and Arabie, 1985) assesses the degree of agreement between two partitions of the same objects. In this study the adjusted rand index was used to compare the grouping based on cluster analysis to known groups (obtained through simulation). We also used the adjusted rand index to study the similarities between groups formed using Ward's method with different genetic distance measures. A brief description of adjusted rand index is given below (detailed mathematical description see Santos and Embrechts, 2009).

Consider a set of *N* objects (or individuals) $S = \{O_1, O_2, ..., O_N\}$ (O_i = object *i*; i =1, 2,...,*N*) and suppose that $U = \{u_1, u_2, ..., u_R\}$ and $V = \{v_1, v_2, ..., v_Q\}$ represent two different partitions (e.g. cluster analysis groupings versus true simulated subpopulations) of the individuals in *S* such that $\cup_{r=1}^{R} u_r = S = \cup_{q=1}^{Q} v_q$ and $u_r \cap u_{r'} = \phi = v_q \cap v_{q'}$ for $1 \le' r \ne r' \le R$ and $1 \le q \ne q' \le Q$. Given two different partitions *U* and *V*, with *R* and *Q* subsets, respectively, the contingency table can be formed to indicate group overlap between *U* and *V* (see Table 1).

Table 1: Contingency table for comparing partitions *U* and *V*

Partition*				*V*		
	Group	v_1	v_2	...	v_Q	Total
	u_1	t_{11}	t_{12}	...	t_{1Q}	$t_{1.}$
U	u_2	t_{21}	t_{22}	...	t_{2Q}	$t_{2.}$
	⋮	⋮	⋮	⋮	⋮	⋮
	u_R	t_{R1}	t_{R2}	...	t_{RQ}	$t_{R.}$
Total		$t_{.1}$	$t_{.2}$	...	$t_{.Q}$	$t_{..} = N$

* t_{rq}, represents the number of individuals that were classified in the r^{th} subset of partition *U* and in the q^{th} subset of partition *V*.

The total number of possible combinations of pairs $\binom{N}{2}$ from a given set of individuals can be divided into in four different types of pairs:

$g_1 = \left(\sum_{r=1}^{R}\sum_{q=1}^{Q} t_{rq}^2 - N\right)/2$ - number of pairs of individuals placed in the same group by using methods *U* and *V* (e.g. using cluster analysis and according to the groups used for simulating the marker data);

$g_2 = \left(\sum_{r=1}^{R} t_{r.}^2 - \sum_{r=1}^{R} \sum_{q=1}^{Q} t_{rq}^2 \right) / 2$ - number of pairs of individuals put in same group by method U and different groups by methods V;

$g_3 = \left(\sum_{q=1}^{Q} t_{.q}^2 - \sum_{r=1}^{R} \sum_{q=1}^{Q} t_{rq}^2 \right) / 2$ -number of pairs of individuals that are put in the same group by method V but in different groups by method U and, finally,

$g_4 = \binom{N}{2} - g_1 - g_2 - g_3$ - number of pairs of individuals that are put in different groups by both methods U and V.

The rand index R_index is given by

$$R\_index = \frac{g_1 + g_2}{\binom{N}{2}},$$

Hubert and Arabie (1985) introduced the correction for chance so that the expected value of the rand index is zero for random partitions.

The adjusted rand index (AR_index) is given by

$$AR\_index = \frac{R\_index - E(R\_index)}{Max(R\_index) - E(R\_index)} = \frac{\binom{N}{2}(g_1 + g_4) - [(g_1 + g_2)(g_1 + g_3) + (g_3 + g_4)(g_2 + g_4)]}{\binom{N}{2}^2 - [(g_1 + g_2)(g_1 + g_3) + (g_3 + g_4)(g_2 + g_4)]},$$

where $E(R\_index)$ represents the expected value of *R_index* and $Max(R\_index)$ the maximum value of *R_index* (see Hubert and Arabie, 1985 for details); AR_index has expectation zero and maximum 1.

Correlation between the group membership matrix and genetic distances

This correlation is used to determine the effect of the number of PC (used for calculating distances) on the distortion of relationship between individuals both within and between groups. Elements of the group membership matrix are either one (if two accessions belong to the same population) or zero (if two accessions belong to different populations). This correlation was only used for the simulated data when group membership is known exactly .

3.3 Results:

3.3.1 Coconut data

A PCA of the coconut data reveals the presence of significant population structure. The total number of significant PC is 14, explaining 21.3 percent of variance. The correlation between Euclidean distances based on the first 14 PC with other genetic distances ranged from 0.512 (with simple matching) to 0.700 (with proportion of shared alleles). Distances based on SSR coded as binary markers (Jaccard and simple matching) are highly correlated (0.884) among themselves but also with distances based on the proportion of shared alleles (0.869 and 0.919 with simple matching and Jaccard, respectively).

Clustering using Ward's method and model-based clustering using the first 14 PC resulted into 15 groups which coincided well with the region of origin of the accessions (see Table 2). The large group of Pacific accessions (PCF) were clustered into seven groups (six for model-based clustering), whereas accessions from Central America (CA) and South East Asia were each clustered into two groups. Accessions from West Africa (WA), Latin America (LA), South Asia and East Africa each formed one group, with both Ward's method and model-based clustering. The South East Asian Dwarf accessions (SEA2) also formed a single cluster with Ward's method but two with model-based clustering. Analysis of the seven groups of accessions from the Pacific formed by Ward (using Euclidean distance) showed good associations with specific islands or groups of islands. For example, the largest group (135) has 80% of the accessions coming from Papua New Guinea; accessions from the South Pacific (Fiji, Cook Island, Tonga and French Polynesia) form one group, accessions from the Mid Pacific (Marshall Islands, Kirivatu and Tuvalu) form their own group too (see Appendix 1).

Groups formed with Ward's method using other distance measures (proportion of shared alleles, Jaccard and simple matching) differ from groups formed with Ward's method using Euclidean distances (based on the 1^{st} 14 PC) in the grouping of accessions from the Pacific, South Asia and South East Asia. It can be seen from Table 2 that accessions from South Asia and South East Asia form more than one group when clustering is done using Ward's method with distances based on proportion of shared alleles. For the accessions from the Pacific Islands the correspondence between the islands as origins of accessions and the groups formed by Ward's method with distances based on the proportion of shared alleles distances is poor (see Appendix 1). For example, accessions from Papua New Guinea and Vanuatu no longer form their own separate groups when clustering is done using Ward's method with distances based on the proportion of shared alleles.

The level of agreement between groups formed by Ward's method using different distance measures depends on the number of groups the accessions are clustered into. When the dendrogram is cut into two or three groups (major groups in the data), all values of the adjusted rand index are above 0.96. However, when dendrogram is cut into fifteen clusters (number of clusters predicted by the TW test), the agreements between the groups formed using different distances are considerably smaller (values range from 0.468 to 0.771) (see Table 3).

Table 2: The distribution of accessions from different origins into the 15 clusters formed using Ward's method with Euclidean distance (14 PCs) and, distance based on proportion of shared alleles and model-based clustering . Cluster numbering is arbitrary

	Euclidean distances based on 1^{st} 14 PCs (E-r)														
Origin[1]	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CA	80	23	-	-	-	-	-	-	-	-	-	-	2	-	-
CAR	-	-	-	-	-	-	-	-	-	-	-	2	-	-	2
EA	-	-	115	-	-	-	-	-	-	-	-	9	-	-	-
LA	-	-	-	69	-	-	-	-	-	-	-	-	1	-	2
NAM1	-	-	-	-	2	3	9	-	-	-	-	-	29	-	-
NAM2	-	-	1	-	-	-	-	-	-	-	-	8	-	-	-
PFC	-	-	-	-	135	18	114	27	15	20	41	-	3	3	-
PFC2	-	-	-	-	2	-	-	-	-	-	-	-	-	2	-
SA	-	-	7	1	1	-	-	-	-	-	-	41	-	3	9
SEA	-	-	1	-	13	5	-	-	-	-	-	5	115	4	-
SEA2	-	-	-	-	-	-	-	-	-	-	-	-	5	35	-
WA	-	-	-	-	-	-	-	-	-	-	-	-	-	3	29
Model-based clustering using 1^{st} 14 PCs (MC)															
Origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CA	79	23	1	-	-	-	-	-	-	-	-	-	1	1	-
CAR	-	-	-	-	-	-	-	-	-	-	3	-	-	-	1
EA	-	-	116	-	-	-	-	-	-	-	8	-	-	-	-
LA	-	-	6	63	-	-	-	-	-	-	3	-	-	-	-
NAM1	-	-	-	-	-	1	9	1	-	-	-	-	31	1	-
NAM2	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-
PFC	-	-	-	-	111	65	115	14	25	36	-	3	5	2	-
PFC2	-	-	-	-	1	-	-	-	-	-	-	2	-	1	-
SA	-	-	7	2	-	-	-	-	-	-	49	1	-	2	1
SEA2	-	-	-	-	-	-	-	-	-	-	-	22	1	17	-
SEA	-	-	3	-	5	7	2	-	-	-	5	1	104	16	-
WA	-	-	-	2	-	-	-	-	-	-	6	-	-	3	21
Distances based proportion of shared alleles (PS)															
Origin	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
CA	102	1	-	-	-	-	-	-	-	-	-	1	-	1	-
CAR	-	-	1	-	-	-	-	-	-	-	-	-	-	-	3
EA	-	116	-	-	-	-	-	-	-	2	6	-	-	-	-
LA	-	5	66	-	-	-	-	-	-	-	1	-	-	-	-
NAM1	-	-	-	1	-	7	-	-	-	6	-	1	-	28	-
NAM2	-	-	-	-	-	-	-	-	-	-	9	-	-	-	-
PFC	-	1	-	85	78	69	24	14	32	61	-	7	3	2	-
PFC2	-	-	-	-	-	-	-	-	-	2	-	-	2	-	-
SA	-	12	-	-	-	-	-	-	-	-	30	-	3	-	17
SEA	-	6	-	1	3	3	-	-	-	27	-	54	10	39	-
SEA2	-	-	-	-	-	-	-	-	-	-	-	1	39	-	-
WA	-	-	-	-	-	-	-	-	-	-	-	-	3	-	29

[1] CA: Central America (Panama), CAR: Caribbean, EA: East Africa, LA: South America (Brazil), NAM1: North America (Pacific), NAM2: North America (Atlantic), PFC: Pacific Islands, PFC2: Pacific Islands (dwarf), SEA: South East Asia, SEA2: South East Asia (dwarf), WA: West Africa

Table 3: Adjusted rand index showing the agreement between groups formed by Ward's method using different genetic distances. The groups are formed by cutting the dendrogram into 2, 3, 4 and 15 clusters. The agreement between groups formed by different distance measures reduces when the number of clusters increases

	Two clusters				Three clusters			
E-r[1]	1.000				1.000			
PS	0.968	1.000			0.974	1.000		
Jaccard	0.984	0.976	1.000		0.983	0.979	1.000	
SM	0.980	0.972	0.980	1.000	0.984	0.975	0.982	1.000
	Four clusters				Fifteen clusters			
E-r	1.000				1.000			
PS	0.693	1.000			0.468	1.000		
Jaccard	0.704	0.965	1.000		0.456	0.500	1.000	
SM	0.880	0.687	0.693	1	0.545	0.578	0.573	1.000
	E-r	PS	Jaccard	SM	E-r	PS	Jaccard	SM

[1] E-r - Euclidean distance based on the first *k*-1 PCs, PSA: distance based on proportion of shared alleles, SM: simple matching distance

3.3.2 Simulated data

Relationship between different genetic distances

Euclidean distances based on the first *k*-1 PC (*k* is the number of subpopulations in the data) have a low to moderate correlation with the other genetic distances (proportion of shared alleles, Jaccard and simple matching). This correlation increases with the strength of subgroup differentiation (see Fig 1). The distances based on SSR markers coded as binary markers (Jaccard and simple matching) are highly correlated (on average 0.917) among themselves and this correlation is less affected by the strength of subgroup differentiation. Distances based on proportion of shared alleles also have a high correlation with distances based on SSR coded as binary markers (on average 0.876 and 0.845 with Jaccard and simple matching, respectively).

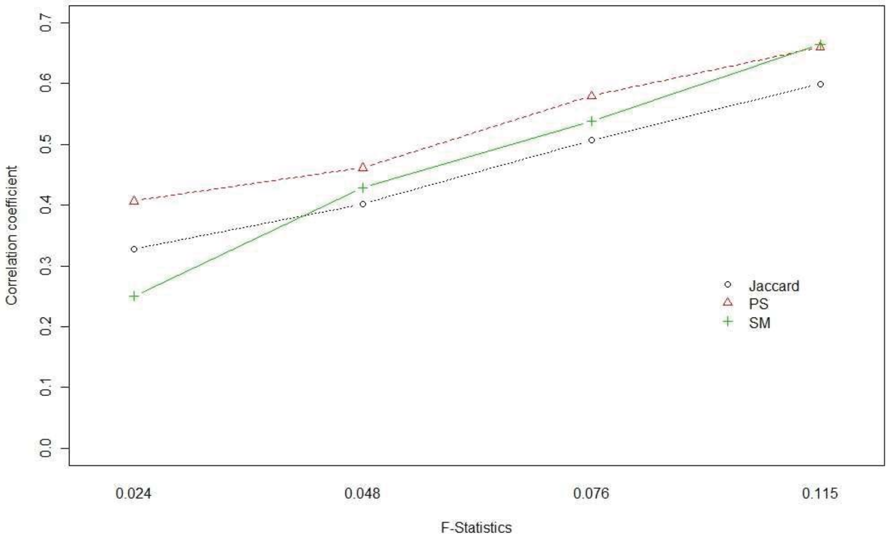

Fig 1: A plot of the correlations between Euclidean distances based on the first *k*-1 PC (*k* is the number of subpopulations in the data) and other genetic distances (PSA: distances based on the proportion of shared alleles, SM: simple matching distance) against the population differentiation (F_{ST}) for a data set with five (k = 5) subgroups.

Clustering success (Cluster purity and Adjusted Rand Index)

The ability of Ward's method to recover the original groups in the data (based on cluster purity and adjusted rand index) increases with the number of PC used for calculating distances between accessions before levelling off at the first *k*-1 PC (see Fig 2). For data sets with the highest number (15) of subgroups, the levelling off takes place at a number of PC less than *k*-1 when subgroup differentiation is high. The increase in cluster purity and adjusted rand index was also observed for model-based clustering (result not shown). However for model-based clustering, as the number of PCs increases beyond *k*-1 the results (cluster purity and adjusted rand index) become more erratic.

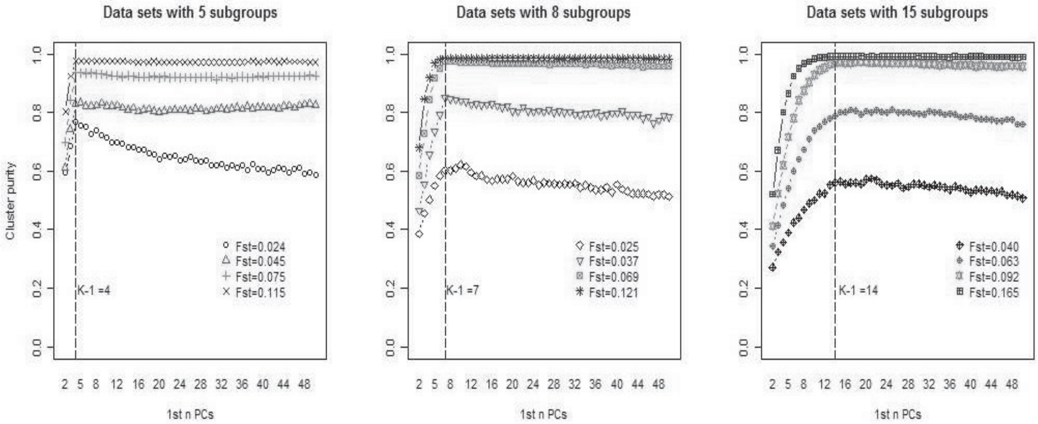

Fig 2: Plot of cluster purity verses the number of PC used for performing cluster analysis using Ward's method for simulated data with different number of subgroups (5, 8 and 15) and different levels of genetic differentiation (F_{ST}).

Clustering with Ward's method using Euclidean distances calculated using the first *k*-1 PC performs much better than clustering using other genetic distances especially at low levels of subgroup differentiation. Results of Ward's method using the first *k*-1 PC are similar to those of model-based clustering for all levels of subgroup differentiation. Euclidean distances based on all PC produced the worst results (see Fig 3).

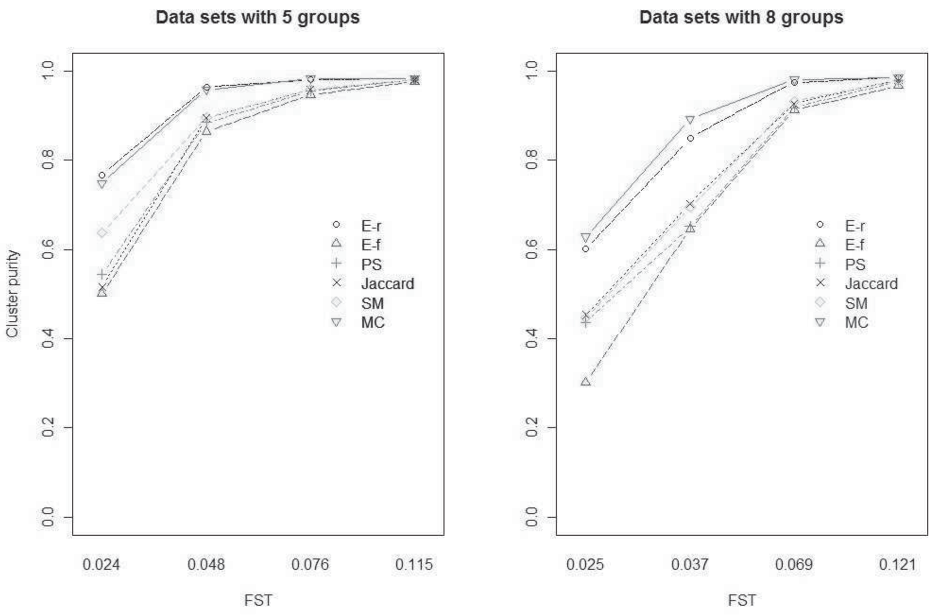

Fig 3: Plot of cluster purity for Ward's dendrograms based on different genetic distances (E-r: Euclidean distance based on the first *k*-1 PC; E-f: Euclidean distance based on all PC; PS: distance based on the proportion of shared alleles; SM: simple matching distance) and MC: model-based clustering

For all the simulated data, the similarity (adjusted rand index) between groups formed by Ward's method using different genetic distances increases with subgroup differentiation (Table 4).

Table 4: Adjusted rand index showing the agreement between groups formed by Ward's method using different distance measures (for simulated data with 8 groups). The groups are formed by cutting the dendrogram into 8 clusters. Agreement increases with subgroup differentiation

	F_{ST}=0.025				**F_{ST} =0.037**			
E-r[1]	1.000				1.000			
PS	0.106	1.000			0.323	1.000		
Jaccard	0.113	0.093	1.000		0.375	0.265	1.000	
SM	0.109	0.091	0.132	1.000	0.369	0.264	0.356	1.000
	F_{ST} =0.069				**F_{ST} =0.121**			
E-r	1.000				1.000			
PS	0.810	1.000			0.957	1.000		
Jaccard	0.827	0.763	1.000		0.963	0.953	1.000	
SM	0.838	0.772	0.817	1.000	0.963	0.956	0.963	1.000
	E-r	PS	Jaccard	SM	E-r	PS	Jaccard	SM

[1] E-r – Euclidean distances based on the first *k*-1 PC, PS: distance based on the proportion of shared alleles, SM: simple matching distance

Correlation between the group membership matrix and observed distances

Ward's clustering using Euclidean distances based on the first *k*-1 PC produces clusters with the highest within-group similarities and the highest between-group dissimilarities, i.e. clusters formed are of very high resolution (see Fig 4). A marked relationship between the number of the first *n* PC used for cluster analysis and the quality of clusters is a clear indication of the importance of using the right number of PC when performing cluster analysis.

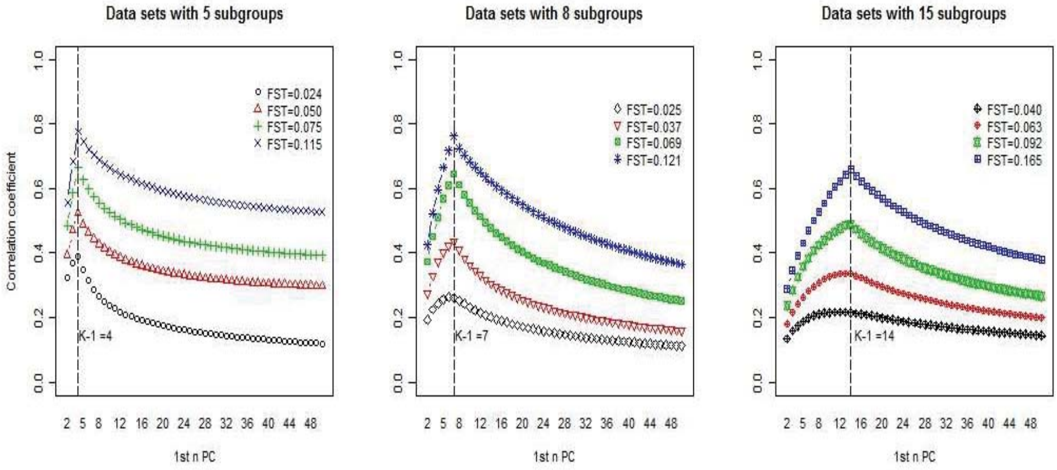

Fig 4: Plot of correlations between the group membership matrix and Euclidean distances based on the first *n* PC for simulated data sets with different numbers of subgroups and different levels of subgroup differentiation (F_{ST}) . Group membership is based on simulated groups.

Determination of the number of groups (clusters)

The ability of the TW test to determine appropriate numbers of subgroups depended on the number of subgroups in the data as well as the strength of subgroup differentiation. For moderate to highly differentiated subgroups, the TW test using a significance threshold of 0.01 performed well in identifying the correct number of subgroups for simulated data sets with 8 and 15 groups (see Fig 5). For data sets with five and eight groups the TW test sometimes overestimates the number of subgroups slightly. Except for very low population differentiation (F_{ST} =0.024, 0.025 and 0.040), when Ward clustering is done using Euclidean distance based on significant PC (even when TW test failed to predict the true number of subgroups), ASC and PBC always identified the correct number of groups (see Fig 6). When model-based clustering was done using significant PCs, the clustering process converge on the right number of clusters except for data sets with low subgroup differentiation (result not shown).

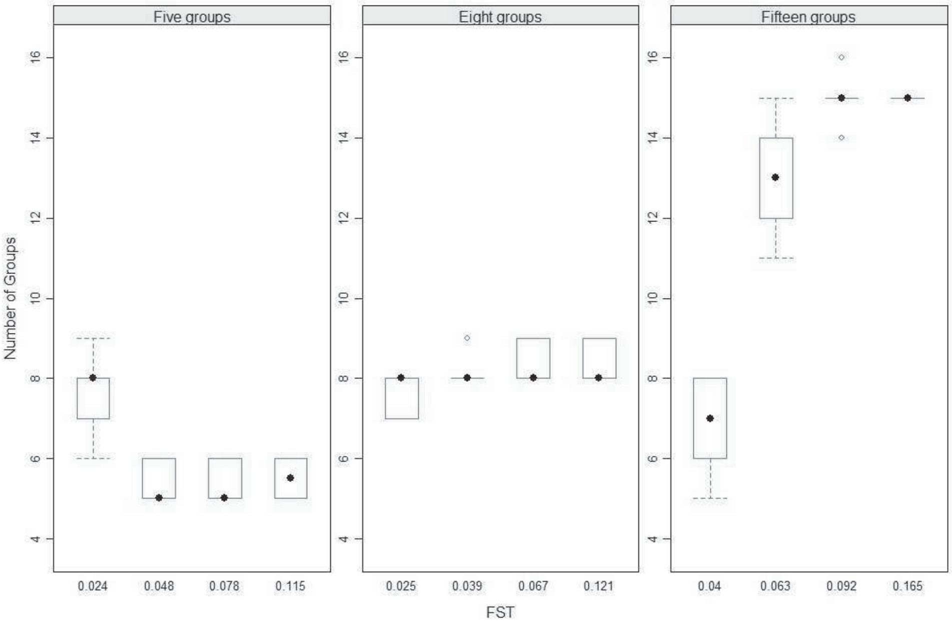

Fig 5: Distributions of the number of groups determined using Tracy-Widom distribution (test) for data sets with different numbers of groups (5, 8 and 15) and different levels of population structure (F_{ST}). Box plots are based on 20 data sets with similar F_{ST} values .

When other distance measures (proportion of shared alleles, Jaccard and Simple matching) were used in combination with Ward's clustering there were no differences in the performance of the two criteria (ASC and PBC) for determining the number of clusters.

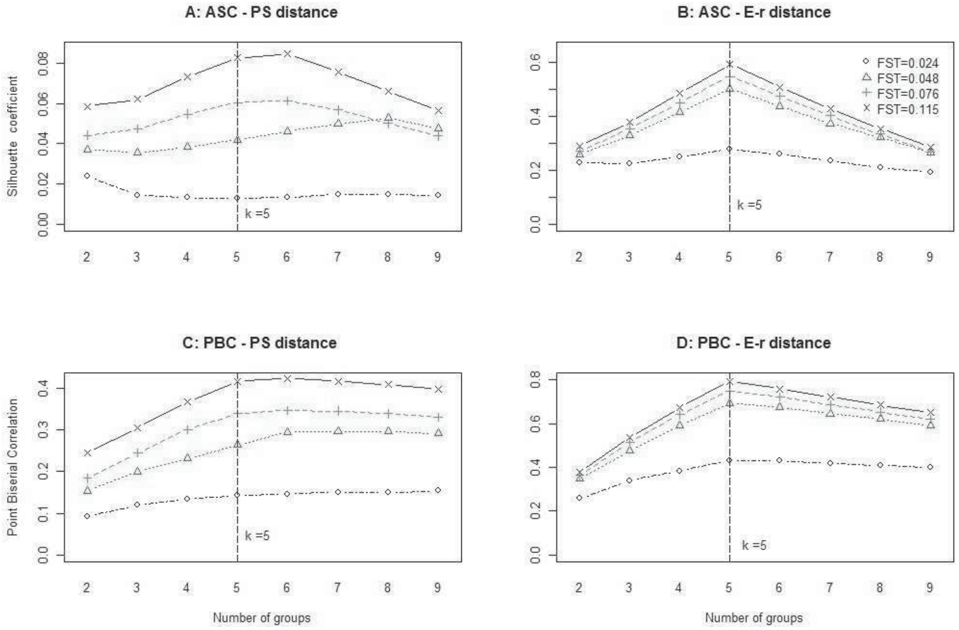

Fig 6: Plot of Average Silhouette Coefficient (A and B) and Point Biserial Coefficient and (C and D) versus the number of clusters obtained by cutting Ward's dendrograms (for data sets with five groups; k=5). A and C obtained by clustering distance based on proportion of shared alleles (PS) while for B and D clustering was done using Euclidean distance (using significant PCs – E-r). For both criteria, the peak or the point at which the graphs start levelling off at is the right number of clusters. Each point is based on average from 25 data sets of similar F_{ST}.

3.4 Discussion

In this paper we have shown that cluster analysis in conjunction with PCA can be a very useful combination of tools for studying the genetic structure of heterogeneous germplasm collections. By emphasizing differences between populations, PCA provides a good description of the genetic structure (McVean2009; Patterson et al 2009). It is clear from this study that the identification of the correct number of PC to be used in cluster analysis is very important. Although there are many statistical criteria for deciding on the optimum number of clusters (Milligan and Cooper, 1985), their performance with real data from germplasm collections is not always good (Odong et al. 2011). Our simulations show that testing the significance of the eigenvalues against the TW distribution, as pioneered by Patterson et al. (2006), works well for SSR data as long as each locus is properly normalized (van Heerwaarden et al. 2010). The decision on the number of PC to be included can therefore be based on a statistic that has a direct population genetic interpretation. We have also noted that sometimes the number of significant PC (based on TW test) over- or underestimates the number of groups in the data. In our simulation study, for data sets with $F_{ST} > 0.05$ the difference between the numbers of significant PC and the expected number (k-1) is only one. An earlier study performed using SNP (Lee et al. 2009) showed a very big difference (> 60) between the number of significant PC and the number of groups in the data. For simulated data, we noted that even in cases where the TW test failed to identify the correct number of groups in the data, performing cluster analysis (with both Ward's method and model-based clustering) using significant PC we are still able to recover the groups well. It is also worth noting that the performances of other methods for determination of the number of clusters (ASC and PBC) are highly improved when clustering is based on significant PC only.

In our simulated data, PCA-based clustering outperformed clustering using other distances in terms of recovering groups from the data. This is mainly due to the fact that PCA emphasizes between-population differences (McVean 2009; Patterson et al. 2009) while smoothing out within-population differences. The high correlation between Euclidean distances based on k-1 PC and the group membership matrix is a clear indication of the effect of PCA in elucidating between-population differences while smoothing within-population differences.

For the coconut data -, the number of groups (15) predicted by TW test is far larger than the number of groups (2) suggested by STRUCTURE and Ward's method using distances based on proportion of shared alleles (see Odong et al. 2011). We speculate that this increase in the number of groups detected is a result of a reduction in noise leading to a better detection of subgroups within the two large groups (Pacific versus Indian and Atlantic Ocean accessions) detected earlier. The 15 groups appear to be quite reasonable since they coincide nicely with the origin of the accessions.

Not surprisingly, the high correlations between genetic distance measures could not always be translated into a high levels of agreement between the groups formed by cluster analysis using those distance measures, especially for data with low levels of subgroup differentiation. The main reason for this is that the correlation coefficient mainly reflects the major group structure in the data and ignores finer details. For real data we noted that although the correlation between Euclidean distance based on *k*-1 PC and other genetic distances are relatively low (0.512 - 0.700) compared to correlations between binary based distance measures (0.884), the major groups in the data were captured well with Ward's clustering using all genetic distance measures. However, cutting the dendrogram into many groups (4 or more), the different genetic distance measures produce groups with low level of agreement (low adjusted rand index). In all cases, Ward's method using Euclidean distance based on significant PC, produces groups that are much more similar to those produced by model-based clustering. In terms of recovering groups in the data, the performances of the distance measures based on SSR markers coded as binary markers are similar. The handling of SSR markers as binary markers has been criticized by Kosman and Leonard, (2005) who proposed a distance similar to distance based on proportion of shared alleles. According to Kosman and Leonard (2005) for diploid organisms, coding alleles as 0 or 1 and using common measures of dissimilarity do not result into an adequate assessment of the genetic dissimilarity between homozygous and heterozygous individuals. They pointed out that for a locus with four alleles A, B, C and D, there is no justification why the distance between genotypes AA and AB should not be the same as the distance between genotypes AB and AC since in both pairs the genotypes only have one allele in common. Both Jaccard and simple matching result into different distances ($\sqrt{1/2}$ versus $\sqrt{3/4}$). The distance based on proportion of shared alleles does not have the above problem. However for practical purposes of grouping of accessions, these differences are not a problem because the interest is only to recover the major groups in the data. However, for a better unraveling of the details of genetic structure, Euclidean distance based significant PC should be preferred. One weakness for the use of Euclidean distance based on only significant PC is that it smoothens out within-group relationships between accessions.

We have shown that appropriately accounting for population structure using PCA, the ability of both hierarchical clustering methods such as Ward's method and model-based clustering to recover subgroups in the data is highly improved.

Appendices

Appendix A: The distribution of accessions from different Pacific Island (origins) into the 7 clusters formed with Ward's method using Euclidean distances (the first 14 PCs). Cluster numbering is arbitrary

	Origins of accessions (Pacific Islands)[1]										
clusters	PNG	VUT	NCL	COK	FJI	PYF	TON	SLB	KIR	MHL	TUV
1	**108**	21	2	0	0	1	0	2	0	0	1
2	1	12	0	0	0	1	0	2	2	0	0
3	9	**94**	**4**	0	1	2	1	3	0	0	0
4	1	2	0	**5**	**10**	**5**	**4**	0	0	0	0
5	0	4	0	0	0	0	0	**11**	0	0	0
6	**19**	0	1	0	0	0	0	0	0	0	0
7	0	1	0	0	0	0	0	0	**19**	**5**	**16**

Appendix B: The distribution of accessions from different Pacific Islands (origins) into the 6 clusters formed using Model-based clustering (on the first 14 PCs). Cluster numbering is arbitrary

	Origin of accessions (Pacific Islands)[1]										
Clusters	PNG	VUT	NCL	COK	FJI	PYF	TON	SLB	KIR	MHL	TUV
1	**99**	7	3	0	0	1	0	0	0	0	1
2	3	**24**	1	**5**	**11**	**8**	**5**	2	6	0	0
3	**14**	**93**	2	0	0	0	0	6	0	0	0
4[2]	4	0	0	0	0	0	0	0	1	0	0
5	0	4	0	0	0	0	0	**10**	0	0	0
6	**21**	3	1	0	0	0	0	0	0	0	0
7	0	1	0	0	0	0	0	0	**14**	**5**	**16**

Appendix C: The distribution of accessions from different Pacific Island (origins) into the 7 clusters formed with Ward's method using distances based on proportion of shared alleles. Cluster numbering is arbitrary

	Origin of accessions (Pacific Islands)[1]										
Clusters	PNG	VUT	NCL	COK	FJI	PYF	TON	SLB	KIR	MHL	TUV
1	67	8	1	0	0	0	0	0	2	0	0
2	0	2	0	4	10	4	4	0	0	0	0
3	37	16	0	1	0	3	0	0	4	0	0
4	12	57	5	0	1	2	1	3	3	1	0
5	18	47	1	0	0	0	0	3	0	0	0
6	0	2	0	0	0	0	0	12	0	0	0
7	0	0	0	0	0	0	0	0	11	4	17

[1] PNG: Papua New Guinea; VUT: Vanuatu; NCL: New Caledonia; COK: Cook; FJI: Fiji; PYF: French Polynesia; TON: Tonga, SLB: Solomon Islands; KIR: Kirivatu; MHL: Marshall Islands and TUV: Tuvalu

Chapter 4

Statistical techniques for defining reference sets of accessions and microsatellite markers

Abstract

Exploitation of the available genetic resources around the world requires information about the relationships and genetic diversity present among genebank collections. These relations can be established by defining for each crop a small but informative set of accessions, together with a small set of reliable molecular markers, that can be used as reference material. In this study, various strategies to arrive at small but informative reference sets are discussed. For selection of accessions, we proposed Genetic Distance Optimization method (GDOpt), which selects a subset of accessions that optimally represent the accessions not included in the core collection. The performance of GDOpt was compared with Core Hunter, an advanced stochastic local search algorithm for selecting core subsets. For the selection of molecular markers, we evaluated a) backward elimination method (BE) and b) methods based on principal component analysis (PCA). We examined the performance of the proposed methodologies using five real datasets. Relative to average distance between an accession and the nearest selected accession (representativeness), GDOpt outperformed Core Hunter. However, Core Hunter outperformed GDOpt with respect to allelic richness. The BE performed much better than other methods in selecting subsets of markers. Methods based on PCA showed that, for practical purposes, the inclusion of the first few (two or three) PCs was often sufficient. In order to obtain robust and high-quality reference sets of accessions and markers we advise a combination of GDOpt (for accessions) and BE or methods based on principal component analysis using a few PCs (for subsets of markers).

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4.1 Introduction

Plant genetic resources stored in genebanks offer great opportunities for improving and securing crop production, especially in marginal environments. Exploitation of the full potential of all available genetic resources around the world requires knowledge about the relationships relative to genetic diversity among genebank collections stored in different centers. The relations between genebank collections can be established by defining for each crop a small but informative set of accessions, together with a small set of reliable molecular markers, that can be used as reference material. Hereafter, the reference material will be referred to as "reference sets."

A reference set of a crop should be an adequate representation of the genetic diversity of that crop as stored in genebanks around the world. In that case, markers can be used to place new accessions in the spectrum of current accessions. The reference sets can also be used to connect different population genetic and quantitative genetic studies, including association studies.

To obtain reliable reference sets, large numbers of accessions have to be genotyped with markers. Under the auspices of the Generation Challenge Programme (GCP; http://www.generationcp.org), large numbers of accessions of important agricultural crops were genotyped with 15 to 50 microsatellite markers. The GCP is a broad network of partners from international agricultural research institutes and national agricultural research programs collectively working to improve crop productivity in the developing world, especially environments prone to drought and having low soil fertility, and high incidences of pests and diseases.

The general philosophy underlying the current study is that molecular markers, such as microsatellites, can be used to represent accessions as points in a multi-dimensional genetic space. A strategy for selecting accessions may consist of choosing accessions in such a way that the whole of the original genetic space is covered by a pre-defined number of accessions. With regard to molecular markers, the reference set should be able to approximate the full genetic space by preserving the distances between the accessions. It may be useful to identify clusters of accessions and use them as a basis for choosing accessions in a stratified way. In addition to statistical principles, molecular genetic requirements should be taken into account, especially the ease of generating markers and marker quality.

The concept of reference sets of accessions and markers is quite similar to the concept of forming core collections using marker information. The reference sets, unlike core collections, place emphasis on the selection of both accessions and molecular markers. In this case, the selected accessions are not linked to a specific genebank collection but taken from collections assembled from many centers. Brown (1995) referred to such a subset of accessions as synthetic core.

In this paper, various strategies to arrive at small but informative reference sets will be discussed. For selection of accessions, we propose a method based on optimization of the spacing of a fixed number of accessions within the genetic space; this method will be referred to as Genetic Distance Optimization method, hereafter referred to as GDOpt. To the best of our knowledge, currently, no method exists for the selection of core collections that aims at obtaining a set of entries to maximize the representation of the accessions in the whole collection. Compared to GDOpt, most existing algorithms for selection of core collections (e.g., Mstrat (Gouesnard et al., 2001), PowerCore (Kim et al. 2007) and Core Hunter (Thachuk et al. 2009)) pay more attention to the content of the core collections but tend to ignore the relationships between the selected entries and those not included in the core collection. The D-method (Franco et al. 2006) maximizes the representation of the groups with the assumption that the groups are known. The GDOpt aims specifically at the selection of core entries that optimally represent accessions not included in the core collection. For the selection of molecular markers, we examined a) a backward elimination method and b) methods based on principal component analysis (PCA). Section 4.2 (Materials and methods) contains a description of the proposed methods and of five datasets used for illustration in this paper. In section 4.3 (Results), the results of the application of the proposed methodologies to five datasets will be presented.

4.2 Materials and Methods

4.2.1 Data

Coconut (*Cocos nucifera*)**:** The coconut data consist of 1014 accessions genotyped with 30 SSR markers. The accessions were collected from different regions of the world (see Table 1) Coconut is a diploid, mainly outcrossing species. Most of the accessions in this collection were described as tall; only 43 dwarf accessions mainly from South East Asia were present. Dwarf coconuts have a high degree of self-fertilization. More than half (19) of the 30 SSR markers used in this study have known positions on the linkage map; they are well-spread across the genome.

Potato (*Solanum* species): The potato data consisted of 233 diploid accessions from four species (*S. ajanhuri* (22); *S. goniocalix* (47); *S. phureja* (105) and *S. stenotomum* (59)) genotyped with 50 SSR markers (see Table 1). Potatoes are mainly outcrossing, with a substantial amount of self-fertilization. The linkage group of 42 of the 50 SSR markers used in study is currently known.

Common bean (*Phaseolus vulgaris*): Genotyped with 36 SSR markers, the common bean dataset consisted of 603 accessions with 296 being described as Andean and 307 as Mesoamerican types (see Table 1). Common bean is a self-pollinating diploid species. Twenty-nine of the 36 SSR markers used in study belong to known linkage groups.

Rice (*Oryza sativa*): The rice dataset consisted of 1998 accessions genotyped with 37 markers (see Table 1). Rice is a self-pollinating diploid species. The linkage map positions of all 37 SSR markers used in study are known.

Chickpea (*Cicer arietinum*): The chickpea data consisted of 3000 accessions genotyped with 50 SSR markers. The accessions originated from more than 60 countries (mainly from the Middle East and other parts of Asia), with germplasm collections maintained at two international centers (ICRISAT in India and ICARDA in Syria) and at several national gene banks (see Table 1). Chickpea is a self-pollinating diploid species. Thirty-two of the 50 SSR markers used in study have known linkage groups but the positions of the markers on the linkage map were not available.

Table 1: Summary information on the five data sets used in this study

Crop	Number of accessions	Origins of Accessions*	Number of SSR markers
Coconut	1014	West Africa(32); North America(52); South Asia(62); Latin America(72); Central America & the Caribbean(109); East Africa(124); South East Asia(183); the Pacific Islands(380)	30
Potato	233	Peru(91); Colombia(80); Bolivia(44); Ecuador(16); Argentina(1) and Chile(1)	50
Common bean	603	Peru(184); Mexico(178); Guatemala(6); Ecuador(1 35); Colombia(29); Brazil(22) and others (18 countries)(94)	36
Rice	1988	India(320); Bangladesh(210); China(167); Indonesia(166); Philippines(139); Liberia(137); Sri Lanka (124); Thailand(122); USA (99); Malaysia (97); Madagascar (87); Nigeria (80) and others (250)	37
Chickpea	3000	India(820); Iran(552); Syria (183); Turkey(160); Afghanistan(147); ICRISAT collections of mixed origin(138), and Ethiopia(124) and others(876).	50

*The numbers in the parentheses indicate the number of accessions from each area of origin

4.2.2 Strategies for selecting representative accessions

A number of strategies for selecting subsets from large collections of accessions (with special reference to the forming of core collections) have been proposed: MSTRAT (Gouesnard et al. 2001), Genetic Distance Sampling (Jansen and Van Hintum, 2007), PowerCore (Kim et al. 2007) and Core Hunter (Thachuk et al. 2009). With the exception of Genetic Distance Sampling, all methods mentioned above apply the M-strategy (Schoen and Brown, 1993) in some way; the M-strategy aims at maximising the number of observed alleles of the markers in the subset of selected accessions. In Genetic Distance Sampling, accessions are selected in such a way that selected accessions are always a pre-defined distance (selection radius) away from each other. This ensures that no duplicates or similar accessions are selected. A disadvantage of the M-strategy is that it is likely to select non-representative accessions ("outliers"). None of the above methods was developed to select accessions to serve as representatives, around which the other accessions can be positioned. In this paper, we propose Genetic Distance Optimization (GDOpt) for selecting representative accessions.

Genetic Distance Optimization:

The aim of GDOpt is to select a fixed number (say *K*) of representative accessions. It is a form of *K*-medoids clustering (Kaufman and Rousseeuw, 1990), in which one accession in each of *K* clusters acts as center of the cluster. Clusters are formed by minimizing the total distance of all accessions to the nearest of the *K* accessions designated as cluster centers. The current algorithm utilizes simulated annealing (Kirkpatrick et al. 1983). To obtain a good starting point, the initial configuration of cluster centers is provided by a modified version of Genetic Distance Sampling (Jansen and Van Hintum, 2007). Genetic distance sampling was modified to select a fixed number of accessions by adjusting the selection radius until the number of accessions selected by genetic distance sampling was equal to or greater than the required size of the reference set. If the number of accessions selected by genetic distance sampling is greater than the intended size of the reference set, random sampling is used to delete the extras. Eventually, the algorithm will be made available as a procedure in the Biometris Genstat Library (http://www.biometris.nl/), but at the moment it is available on request from the authors.

Comparison with Core Hunter:

In this paper, the results obtained with GDOpt are compared with those obtained with Core Hunter (Thachuk et al. 2009). Core Hunter was selected because the authors have demonstrated its superiority over other existing methods of core selection. In Core Hunter, the weights attached to two optimization criteria (Modified Rogers Distance and Shannon Diversity Index) were varied. By assigning all the weight to the Modified Rogers Distance, Core Hunter maximizes the average genetic distance between selected accessions, whereas by assigning all the weight to Shannon Diversity Index, it maximizes the number of alleles in the selected accessions. The comparison was based on two criteria: a) the distance between accessions and the nearest entry in the reference set (representativeness) and b) the proportion of alleles captured in a subset of a specified sample size selected by each method. This comparison was done to show that forming core collections with the intention to maximize either allelic richness or distances between entries (e.g., using Core Hunter settings in this study) compromises the ability to represent the contents of the whole collection.

The results from GDOpt and Core Hunter were also compared with those from simple random sampling (for real data) and stratified random sampling for simulated data. The results for the simulated datasets are presented in Appendix 1.

4.2.3 Selecting subsets of molecular markers

Criterion:

In the current context, the criterion used for comparing different methods of selecting subsets of molecular markers is based on the preservation of genetic distances between accessions. The key assumption is that by preserving genetic distances between accessions, population structure (if present) will be preserved. The criterion applied in all cases is the correlation between genetic distances between accessions based on a subset of molecular markers and genetic distances based on all available markers.

Polymorphism information content (PIC):

The PIC (Botstein et al. 1980) depends on the number and frequencies of alleles. According to this criterion, a marker with many alleles with small frequencies is more informative than a marker with two alleles with equal frequencies. The PIC does not take into account the dependencies between markers. Because it is one of the most frequently used criteria for selecting sets of molecular markers, the performance of other methods will be compared with that based on PIC.

Methods based on Principal Component Analysis (PCA):

These methods use the dimension reduction ability of PCA to identify a subset of molecular markers that should be retained to achieve minimum loss of information. Recently, the use of PCA for selecting subsets of molecular markers (especially single nucleotide polymorphisms (SNPs)) has been discussed by Paschou et al. (2007) and Zhang et al. (2009).

Molecular markers are selected based on the weighted sum of squared loadings on all principal components (PCs) designated as important, using the corresponding eigenvalues as weights. The method will be referred to as 'Weighted principal component analysis WPCA'. The steps are (1) Perform PCA on the accession-by-marker matrix; (2) Decide on the number of PCs to be designated as important; (3) Calculate the weighted sum of squares of the loadings of each marker on the PCs designated as important; (4) The markers are ranked in descending order based on their weighted sums of squared loadings. The molecular markers are then included in the subset based on their ranks. For weighted PCA, we compared (1) ranking based on the first PC (WPCA1); (2) ranking based on the first two PCs (WPCA2); (3) ranking based on the first three PCs (WPCA3) and (4) ranking based on the first 20 PCs (WPCA20) when selecting a subset of markers.

Patterson et al. (2006) discussed the use of the Tracy-Widom distribution for determining the number of significant principal components for SNP data. This is done by comparing standardized eigenvalues with the Tracy-Widom distribution. If n differentiated groups of genotypes are present in the data, one expects to find $k = n$-1 significant eigenvalues. However, in practice it has become standard to designate the first two or three principal components as important and discard the rest without performing any statistical test. Formal testing usually leads to many statistically significant PCs.

Application of PCA to SSR data requires special attention. The SSR markers were first recoded as 0, 1 or 2 based on the number of copies of the allele with frequency closest to 0.5. The advantage of treating SSR markers in this way lies in its simplicity. We expect the loss of information associated with coding SSR markers in this way to be small in most cases. The SSR-marker data were recoded as described above to reduce the information from each SSR marker into a single column, which can then be easily related to PCs.

Backward Elimination (BE):

This method is similar to the backward elimination method used for variable selection in multiple regression. It uses the correlation between the genetic distances (between accessions) based on all molecular markers and the genetic distances based on a subset of markers as the criterion for deleting markers. In a stepwise approach, at each step, the molecular marker whose exclusion leads to the smallest reduction in correlation between the two sets of distances is removed until a specified level of correlation or a desired number of molecular markers is reached.

The BE method can be summarized as follows:
Step 1: Calculate the distances between accessions using all the molecular markers. Let D_0 be the matrix of those distances ($D_0 = (d_{ij})$, where d_{ij} is the distance between accession *i* and *j*.
Step 2: For each of the *m* markers, calculate the distances between accessions by leaving out one marker at a time. Let D_{-e} ($e = 1,2,...,m$) be the matrix of distances between accessions constructed with marker e left out ($D_{-e} = (d_{ije})$, where d_{ije} is the distance between accessions *i* and *j* calculated when marker e is left out). Denote r_{-e} as the correlation between D_0 and D_{-e}.

$$r_{-e} = \frac{\sum_{i<j}^{n}(d_{ij} - \overline{d})(d_{ije} - \overline{d}_{ije})}{\left[\sum_{i<j}(d_{ij} - \overline{d})^2 \sum_{i<j}(d_{ije} - \overline{d}_{ije})^2\right]}$$

Step 3: Select marker with the largest r_{-e} value, and eliminate it (the marker) from the dataset and repeat Step 2 with the remaining markers. Each time, the maximum value of r_{-e} is recorded.

Step 4: Repeat steps 2 and 3 until either the maximum value of r_{-e} reaches the stopping value set or until the desired number of markers is achieved.

Similarity measures

In this paper, we used genetic distances (*D*) based on the proportion of shared alleles (*PSA*) applied to the original SSR marker data and the recoded data, where *D* = 1 - *PSA*, and

$$PSA = \left[\sum_{m=1}^{M}\sum_{a=1}^{A_m}\min(f_{1ma}, f_{2ma})\right] / M \ ,$$

where f_{1ma} and f_{2ma} are the frequencies of allele a (a=1, 2… A_m) for molecular marker m (m=1, 2… M) in individuals 1 and 2, respectively. For more information on the proportion of shared alleles as similarity measure, see Bowcock et al. (1994), Chakraborty and Jin (1994) and Chang et al. (2009).

Other important aspects of selecting subsets of molecular markers

In addition to the statistical criteria used for selecting molecular markers, a number of important issues should also be examined. The non-statistical issues of importance in marker selection are quality relative to clarity and repeatability of banding pattern, ease of automation of allele calling and genome coverage and linkage between markers. The markers selected should be of high quality with highly reproducible alleles.

4.3 Results

4.3.1 Selection of accessions

General results: In the following, representativeness is measured as average distance between each accession to the nearest selected entry in the subset of accessions (Table 2). The GDOpt produces subsets of accessions that are much more representative compared with Core Hunter. In all the crops, the average distance from accessions to its nearest entry in the subset of accessions is smaller for GDOpt compared with all settings of Core Hunter. Random sampling also performed much better than all the different settings of Core Hunter relative to representativeness of the whole collection.

Table 2: Average distances between accessions and their nearest entry in the selected subset of accessions obtained using Genetic Distance Optimization (GDOpt), Random sampling and Core Hunter with five (CH1 – CH5) different parameter settings in terms of Modified Rogers distance (MR) and Shannon diversity index (SH). Random sampling values were obtained from 100 samples

	Crop				
Method	Coconut	Potato	Common bean	Rice	Chickpea
GDOpt	0.389	0.216	0.359	0.472	0.646
Random sampling	0.463	0.274	0.443	0.548	0.729
CH1 (MR=1.0;SH= 0.0)*	0.490	0.307	0.467	0.547	0.760
CH2 (MR= 0.7;SH=0.3)	0.522	0.325	0.476	0.551	0.775
CH3 (MR=0.5;SH=0.5)	0.531	0.327	0.478	0.542	0.760
CH4 (MR=0.3;SH=0.7)	0.527	0.326	0.474	0.534	0.748
CH5 (MR=0.0;SH=1.0)	0.521	0.321	0.483	0.537	0.766

*The values in the parentheses show the different weights given to modified Rogers distance (MR) and Shannon diversity index (SH) used when selecting a subset of accessions using Core Hunter

With regard to the total number of alleles captured by subsets of 15 selected accessions (Table 3), all parameter settings of Core Hunter performed better than GDOpt. However, major differences were found in the retention of alleles with different frequencies (see Fig. 1). For ease of interpretation, we have classified alleles into three categories based on their frequencies (p): a) common alleles-CA ($p \geq 0.05$) b) rare alleles-RA ($0.005 \leq p < 0.05$) and c) very rare alleles-VRA ($p < 0.005$). The proportion of common alleles captured by GDOpt and different settings of Core Hunter were comparable. For all five crops, subsets of 15 accessions selected using GDOpt performed well in capturing common alleles. With the exception of chickpea, subsets selected via GDOpt captured more than 85% of all common alleles. In potato and common bean, subsets of accessions obtained by GDOpt showed a higher frequency of common alleles compared with subsets of accessions obtained by the different settings of Core Hunter. Core Hunter performed much better than GDOpt in capturing rare and very rare alleles. However, with simulated data, GDOpt performed better than Core Hunter with all the weights given to Modified Roger's distance relative to proportion of captured alleles (see Appendix 1).

Table 3: Numbers of alleles in the whole datasets and proportions of alleles in subsets of 15 accessions obtained using Genetic Distance Optimization, Random sampling and Core Hunter with five (CH1 – CH5) different parameter settings in terms of Modified Rogers distance (MR) and Shannon diversity index (SH). Random sample values were obtained from 10 samples

	Crop				
Method	Coconut	Potato	Common bean	Rice	Chickpea
Whole dataset	469	367	1089	566	1605
GDOpt	0.422	0.635	0.255	0.339	0.318
Random sampling	0.430	0.554	0.254	0.344	0.264
CH1 (MR=1.0;SH= 0.0)*	0.388	0.700	0.298	0.426	0.318
CH2 (MR= 0.7;SH=0.3)	0.527	0.796	0.332	0.459	0.338
CH3 (MR=0.5;SH=0.5)	0.563	0.820	0.341	0.466	0.336
CH4 (MR=0.3;SH=0.7)	0.569	0.837	0.346	0.463	0.333
CH5 (MR=0.0;SH=1.0)	0.569	0.839	0.350	0.482	0.343

*The values in the parentheses show the different weights given to modified Rogers distance (MR) and Shannon diversity index (SH) used when selecting a subset of accessions using Core Hunter

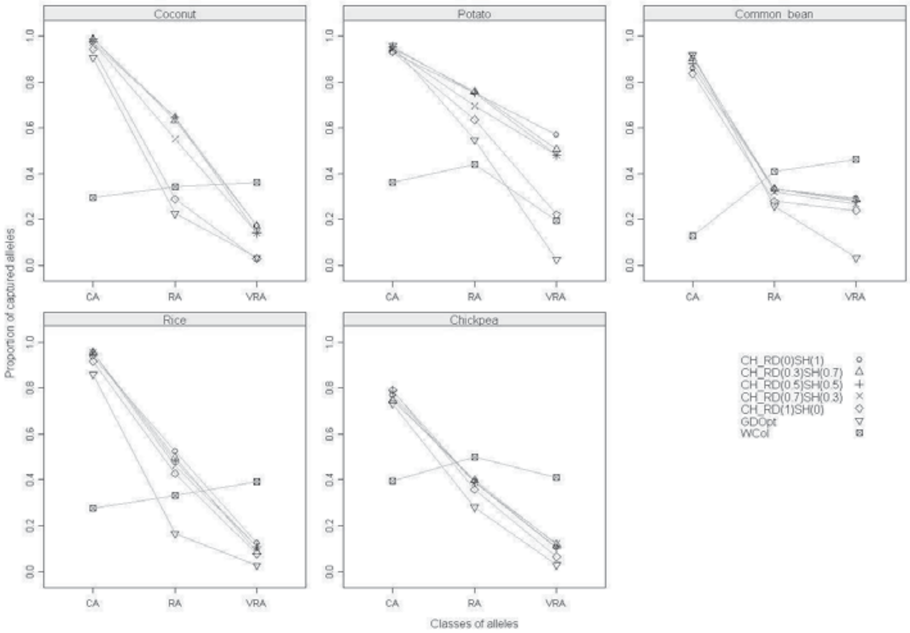


Fig 7: Proportions of alleles in different classes in the whole dataset (Whole_Col) and in subsets of 15 accessions obtained using Genetic Distance Optimization (GOpt), random sampling and different parameter settings for Core Hunter (CH1-RD(1)SH(0); CH2-RD(0.7)SH(0.3); CH3-RD(0.5)SH(0.5); CH4-RD(0.3)SH(0.7); CH5-RD(0)SH(1)). The parameter settings refer to weights assigned to Modified Rogers Distance (RD) and Shannon diversity Index (SH). Classes are based on the frequencies of the alleles in whole collection (Common alleles-CA ($P \geq 0.05$); Rare alleles-RA ($0.005 \leq p < 0.05$) and Very rare alleles-VRA ($p < 0.005$))

4.3.2 Selection of markers

General Results: In the following, the preservation of pairwise distances between accessions by a subset of SSR markers is measured by the correlation between the distances based on the subset of SSR markers and the distances based on the whole set of SSR markers (Table 4).

Table 4: Correlation of pairwise distances between accessions for a subset of five markers versus all the markers with distance based on PSA

	Crop				
Method*	Coconut	Potato	Common bean	Rice	Chickpea
BE	0.813	0.826	0.902	0.706	0.661
WPCA1	0.775	0.718	0.864	0.698	0.640
WPCA2	0.766	0.772	0.722	0.533	0.624
WPCA3	0.653	0.651	0.719	0.407	0.624
WPCA20	0.669	0.607	0.617	0.361	0.535
PIC	0.603	0.663	0.527	0.607	0.347

*BE: Backward Elimination; WPCA1, WPCA2, WPCA3, WPCA20: Weighted Principal Component using the first 1, 2, 3 and 20 PCs, respectively; PIC: Polymorphic Information Content

Across all five crops, BE performed much better than all other methods in selecting a subset of molecular markers for preserving the pairwise distances between accessions. The selection based on PIC performed very poorly in datasets with very many alleles (common bean and chickpea). The method based on WPCA using many principal components (WPCA20) usually produced worse results compared with when one, two or three principal components (WPCA1, WPCA2 or WPCA3) were used. The differences in performance between the methods became more pronounced when selecting small subsets (< 10) of SSR markers (results not shown).

The number of SSR markers required to achieve a specified minimum correlation depended on whether the SSR markers are recoded or not (Table 5). For all five crops, fewer markers were required to achieve a specified correlation when the proportion of shared alleles was calculated from the original SSR data instead of recoded data. The differences can be attributed to the loss of information associated with recoding SSR markers and this loss of information appears to be large for SSR markers with high PIC values.

Table 5: Numbers of selected markers required to achieve a minimum correlation of 0.85 between distances between accessions based on markers selected using different methods and distances between accessions based on all markers. The numbers in the parenthesis is obtained when the distances between accessions were based on SSR data recorded as 0,1 or 2.

	Crop				
Method*	Coconut	Potato	Common bean	Rice	Chickpea
BE	7(12)	6(13)	2(3)	13(18)	16(23)
WPCA1	9(16)	16(23)	2(2)	13(18)	18(26)
WPCA2	9(14)	11(24)	7(11)	13(18)	17(26)
WPCA3	11(15)	15(24)	9(12)	15(19)	17(24)
WPCA20	11(16)	15(28)	11(14)	20(22)	21(25)
PIC	14(20)	18(31)	13(17)	18(22)	40(40)

*BE: Backward Elimination; WPCA1, WPCA2, WPCA3, WPCA20: Weighted Principal Component using the first 1, 2, 3 and 20 PCs, respectively; PIC: Polymorphic Information Content

Evaluation of subsets of five SSR markers indicated that BE and WPCA-based methods tended to select markers whose major alleles had frequencies close to 0.5 (Table 6). These SSR markers separated major groups of accessions. The PIC criterion favored SSR markers with very many alleles. These SSR markers differentiated between individual accessions or small groups of accessions thus played a minimal role in separating major groups.

Table 6: Average frequencies of major alleles in a subset of five SSR markers selected by different methods

	Crop				
Method*	Coconut	Potato	Common bean	Rice	Chickpea
BE	0.501	0.511	0.484	0.311	0.420
WPCA1	0.515	0.566	0.515	0.459	0.435
WPCA2	0.562	0.541	0.320	0.411	0.461
WPCA3	0.388	0.482	0.455	0.440	0.461
WPCA20	0.367	0.428	0.386	0.414	0.209
PIC	0.241	0.357	0.122	0.201	0.070

*BE: Backward Elimination; WPCA1, WPCA2, WPCA3, WPCA20: Weighted Principal Component using the first 1, 2, 3 and 20 PCs, respectively; PIC: Polymorphic Information Content

Crop-specific results

Coconut: The best method for selection of a subset of markers was BE, followed by WPCA1 and WPCA2 (see Fig 2). For example, when distances were based on PSA, using the original SSR marker data we only required seven out 30 markers using BE, compared with 14 using PIC criterion to achieve a correlation of 0.85. The results obtained for WPCA3 and WPCA20 were quite similar to random sampling of marker subsets but better than those for PIC. A similar pattern in the number of molecular markers required to achieve a correlation of 0.85 was observed when distances were calculated using the recoded data, except that the numbers of required markers were much higher.

Potato: BE outperformed all other methods in selecting a subset of molecular markers (see Fig. 2). When pairwise distances between accessions were calculated using PSA based on the original SSR marker data, we only needed six out of 50 markers to achieve a correlation of 0.85, which is less than half the number required by other methods. For the number of molecular markers needed to achieve a correlation of 0.85 (with the exception of WPCA2), the performances of the other methods were quite similar. Only BE, WPCA1 and WPCA2 performed better than random selection.

Common bean: For common bean, a much greater difference in the performance of BE, compared with the other methods (except WPCA1), was found than for the other crops, especially in subsets of markers of small size (see Fig. 2). The PIC performed very poorly in this dataset. The BE and WPCA1 required only two out 33 of SSR markers to achieve a correlation of 0.85 compared with 13 markers for PIC. The performance of WPCA20 was quite similar to that of PIC.

Rice: The BE performed better than the other methods, except WPCA1 (see Fig. 2). The performances of BE, WPCA1 and WPCA2 were very similar for subsets of markers with sizes greater than 10. For subsets of markers of sizes less than 10, random selection of markers performed much better than WPCA3 and WPCA20. With the exception of BE and WPCA1, the method based on PIC performed better than other methods for subsets of size less than 5. When correlation was based on recoded SSR data, WPCA20 and PIC required the same number of markers (22) to achieve a correlation of 0.85.

Chickpea: Although BE method performed better than all the other methods, the differences in performance were not prominent, especially with WPCA-based methods (see Fig. 2). The selection based on PIC performed very poorly compared with the other methods. The PIC required 40 out of 50 markers to achieve a correlation of 0.85. In this case, randomly selecting a subset of SSR markers produced much better results than PIC.

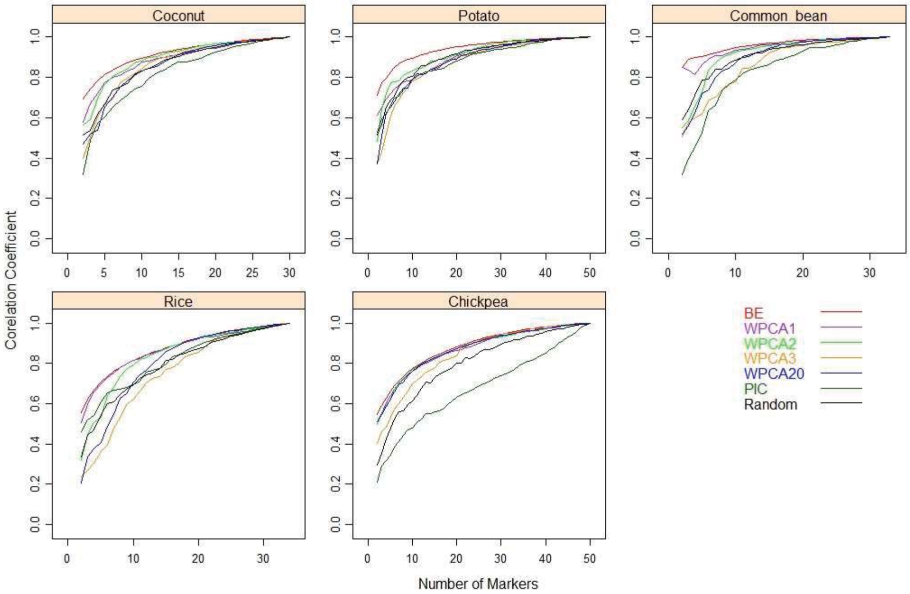


Fig 2: Correlation between distances constructed using a subset of SSR markers versus all the SSR markers for the different selection methods with distances based on PSA

4.4 Discussion and Conclusions

Understanding the current status of genetic diversity and finding links between genetic resources stored in different institutions are essential for a successful, worldwide exploitation of genetic resources for crop improvement. The concept of reference sets of accessions and markers provides an efficient way to relate new materials to existing ones and set up different crop-specific study panels that can be used by plant breeders worldwide, with just a few representative accessions and a few molecular markers covering the genetic diversity in each crop. For example, selected accessions can be used for creating the so-called MAGIC (Multiparent Advanced Generation Inter-Cross) population, which can be used for QTL analysis. Kover et al. (2009) demonstrated the utility of MAGIC population in improving the precision of QTL mapping.

In this study, representative accessions were selected using GDOpt, which aims at optimizing the spacing of a fixed number of accessions within the genetic space defined by all available markers. By performing selection and clustering of accessions simultaneously, this method can avoid the tedious process of determining population structure of the collection. Determination of population structure is quite challenging, especially in the case of germplasm collections where most often no clearly defined groups exist (Odong et al. 2011). In highly diverse collections, it may only be possible to isolate subsets of closely related individuals rather than obtaining large homogenous groups (Hamblin et al. 2007). It is from these closely related individuals that GDOpt selects a representative. Results from simulations have shown that if groups are known, stratified sampling does give improvement over simple random sampling, but its performance is still worse than that of GDOpt (Appendix 1). However, in situations where distinct groups of accessions exist (e.g., the Andean and Mesoamerican types of common beans), the selection can be performed separately for each group. Most methods that aim at optimizing either allelic richness or maximum genetic distances between selected accessions are quite capable of covering the full range of genetic diversity, including extremes, but may not produce representative subsets of accessions. For example, by simply selecting extremes, it would be possible to produce a subset with maximum genetic distances between accessions or maximum number of alleles although the selected accessions are not fully representative of the whole collection. Moreover, according to Zhang et al. (2010), the majority of very rare alleles would not contribute to the genetic diversity needed to develop elite cultivars and therefore their inclusion in the core collection may not be worthwhile. Some scientists (Allard, 1992; Frankel et al. 1995) have argued that less frequent alleles only occasionally affect quality or other traits and are generally unlikely to be of future use. In a situation where a representative subset is required, GDOpt has great advantages over all other methods, as shown in this study.

One of the key challenges in selecting representative sets of accessions based on distances between accessions is the effect of (random) errors in the data. In general, (random) errors will inflate dissimilarities between individuals, with smaller dissimilarities being relatively more inflated than larger ones. The inflation of dissimilarities consequently results in an overall greater dispersion of accessions in the genetic space, making it more difficult to obtain representative sets of accessions. The use of SSR markers with very many alleles (and consequently high PIC values) aggravates this problem. It is thus clear that if we are interested in a stable relationship between accessions, then the distances obtained from all the available markers and/or all alleles may be unsuitable. Markers with very high PIC (or very many alleles), in addition to inflating the distances between accessions, are likely to provide inconsistent relationships because of the fact that some of the alleles are as a result of misreading bands and are not repeatable. A much more stable relationship (distance) between accessions can be obtained by discarding some markers. Our results show that for all the five crops, 10 or more markers can be discarded without much distortion of pairwise distances between accessions. Another alternative for obtaining a stable relationship between accessions or group of accessions would be to calculate distances using important PCs, but additional studies are needed.

For the selection of subsets of molecular markers, we have shown that if one is interested in selecting a subset that preserves pairwise distances between accessions, BE provides the best option. The BE tends to remove markers with very many alleles and lots of missing values because they tend to contribute less to pairwise distances between accessions. The first markers included in the subset using BE mainly separate the major groups present in the data but could have the weakness of not differentiating well between accessions within groups. For example, for the common bean data, only two markers are required to achieve a correlation of 0.85 and those two markers separate Mesoamerican and Andean types quite well. A similar situation was observed for coconut where the first five markers separated accessions associated with the Pacific Ocean from those associated with Indian and Atlantic Oceans. Simulations (results not shown) indicated that the correlation between pairwise distances between accessions based on a subset of markers and distances based on the entire set of markers depended on the level of group structure in the data. The stronger the group structure, the fewer the number of markers required to preserve the pairwise distances between accessions. The performance of BE could be improved by performing marker selections in two steps, i.e., first perform BE based on the whole dataset and subsequently perform it within the major groups. For rice and chickpea, the difference in performance between BE and other methods was smaller compared to common bean, coconut and potatoes. This could be attributed to nature of group structure present in these datasets. Both multidimensional scaling and cluster analysis showed the presence of strong structure that was consistent with passport data in the three datasets (common bean, coconut and potato), which indicated a large difference between BE and other methods of selection of subset of markers compared to rice and chickpea.

The performances of the PCA-based methods were quite good and in some cases comparable with that of BE. Our study revealed one interesting aspect about the number of important principal components to be included in the selection process. In all our datasets, the first few (1 to 3) principal components appeared to be sufficient. For most datasets, the eigenvalues revealed a big difference between the first two or three principal components compared with the rest; which made the contribution of the later PCs of minor importance. The practice of determining the number of important PCs through rigorous statistical testing most often leads to inclusion of too many principal components, which in turn introduces noise. A recent study by Lee et al. (2009) noted a negative effect of including all significant PCs when performing distance-based cluster analysis.

Subsets of markers selected using PIC performed very poorly in preserving pairwise distances between the accessions, especially with common bean and chickpea. The poor performance with common bean and chickpea could be attributed to the poor quality of the data. Both datasets contained many markers with a very large number of alleles with more than 50% of the alleles having frequencies of less than 0.01 (see Appendix 2 for diversity statistics of the SSR markers for common bean data used in this study). In both crop species, the average frequency of major alleles for the five SSR markers with the highest PIC is much smaller compared with subsets formed by BE and PCA-based method. A large of number of alleles with frequencies of less than 0.01 could be because of poor binning of alleles. The presence of error (random) in the data was thus more likely to affect selection of markers based on PIC compared with BE and WPCA-based methods. The BE and WPCA-based methods (especially

WPCA1, WPCA2 and WPCA3) were more robust for detecting errors because those methods only picked out the key features of the data. Although PIC is the most common criterion used for selection of molecular markers, we have shown in this study that it performed poorly with respect to preserving major relationships between groups of individuals. Because PIC measures genetic diversity within a population, its poor performance with respect to identifying major features in the data is not surprising.

When SSR markers were recoded, the difference in performance between the different methods was smaller compared with the results obtained using the original SSR marker scores. This may be because of a loss of information; forcing alleles into just two categories (allele with frequency closest to 0.5 versus others) tends to smooth out differences between accessions. It is clear from literature that one needs more bi-allelic markers to achieve the same level of genetic distance accuracy as a set of multi-allelic markers, such as microsatellites (see Laval et al. 2002). As noted from the results in this study, recoding affected markers with a high PIC much more than other markers. The correlation between distances between the accessions based on the original SSR markers and distances based on recoded SSR markers indicated some loss of information. The correlations for chickpea, coconut, rice, common beans and potatoes were 0.42, 0.69, 0.71, 0.82 and 0.88, respectively. The low correlation for chickpea (0.42) is an indication that recoding SSR data can sometimes lead to a substantial loss of information, and therefore it should be applied cautiously. Other methods, such as performing PCA on allele frequencies from each SSR marker separately and later combining the information across all markers, can be explored.

One of the key advantages of BE and PCA-based method is that the selected molecular markers are likely to be independent. For PIC, unless sets of markers on which selection is done are known to be independent, there is no guarantee that the selected markers will be independent. For the datasets used in this study, several of the markers provided were on different linkage groups and those for which the positions on the chromosomes were given showed wide spacing between the markers (independence).

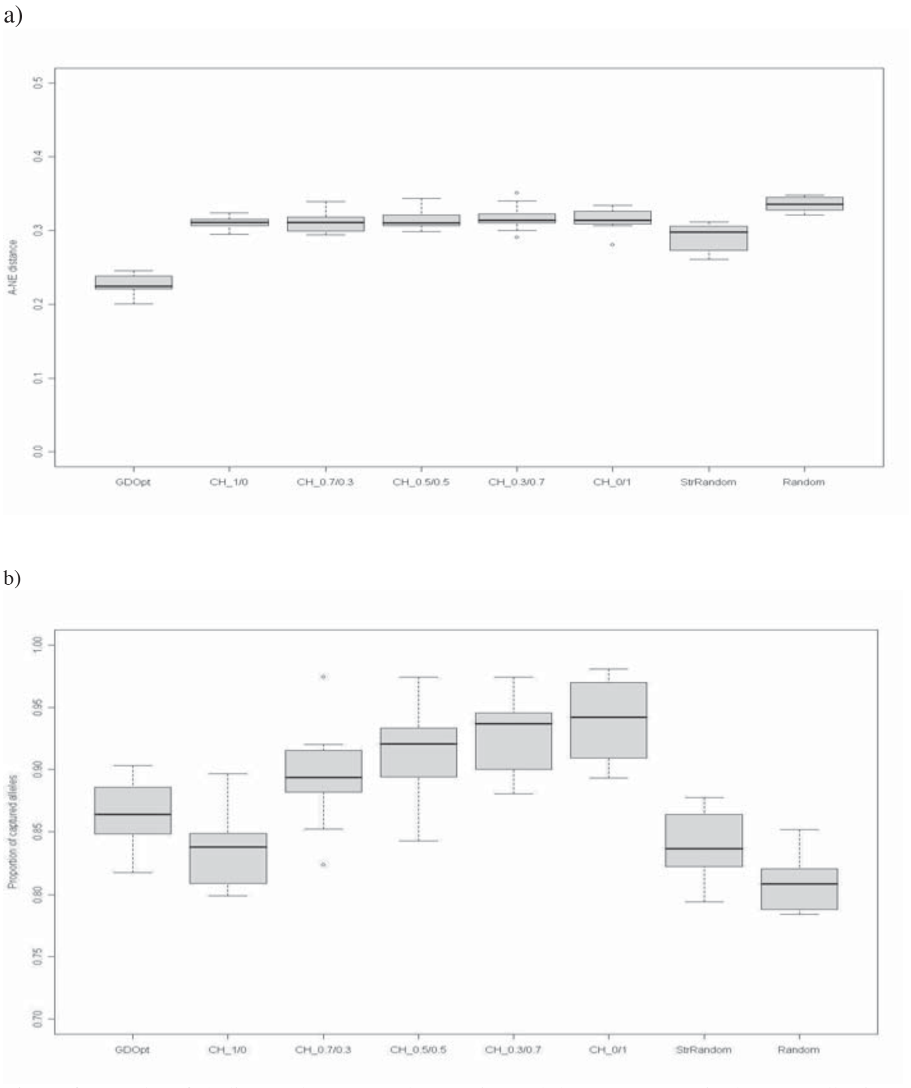
It is clear from our study that using both BE and PCA-based methods, several good subsets of markers can be obtained. Other (quality aspects) of the chosen molecular markers (e.g., the possibilities for multiplexing) can be used to identify the most appropriate set. In the same way, alternative sets of accessions also exist and suitable accessions can be selected to replace less desirable ones. For example, accessions with missing values or those known to have propagation problems can be replaced. Discussion with genebank curators, crop specialists and laboratory technicians can provide information that can be used as a basis of determining which of the selected accessions and molecular markers should be retained or dropped. The use of multivariate statistical techniques, such as multidimensional scaling, can assist in visualizing the selected accession in the space defined by the selected subset of markers.

In summary, for the selection of subsets of both accessions and markers, several methods exist, each with their own advantages and disadvantages, i.e., there is no perfect core collection suitable for all purposes. Although GDOpt performs very well with respect to representativeness of non-selected accessions, its performance with respect to maximizing genetic diversity parameters, such as allelic richness or distances between selected accessions, is slightly compromised – i.e., there is a trade-off. Methods such as Mstrat (Gouesnard et al., 2001) PowerCore (Kim et al. 2007) and Core Hunter (Thachuk et al. 2009) should be used when the interest is in selecting subsets of accessions by maximizing diversity parameters, such as allelic richness or distance between entries in the core collection. For the selection of subsets of molecular markers, both BE and methods based on the first few (two or three) PCs gave rise to subsets of markers that preserved the major structure in the data but may have performed poorly for discriminating between individuals within the groups compared with markers with a high PIC.

Appendices

Appendix 1

Result of simulated data


a)

b)

Figure 3: Boxplot of **a)** distance between each accession and the nearest entry in the core collection (A-NE distance) and **b)** Proportion of alleles captured by core collections (of size 15) obtained by GDOpt, different settings of Core Hunter (CH-0/1, CH-0.7/0.3, CH-0.5/0.5, CH-0.3/0.7, CH-0/1), Random sampling (Random) and Stratified Random sampling (StrRandom) from 10 simulated data sets. For both random and stratified sampling, for each data set sampling was performed 100 times.

Appendix 2

Table 6: Values of diversity statistics for each molecular marker – Common beans

Marker	No. of Alleles	Frequency of Major alleles	No. of Genotypes	Gene Diversity	Heterozygosity	PIC
PV_at001	121	0.0540	181	0.9814	0.6945	0.9810
BM187	95	0.1317	137	0.9533	0.3034	0.9516
GATS91	48	0.0825	79	0.9523	0.0846	0.9502
BM143	65	0.1594	109	0.9394	0.1299	0.9366
BM156	62	0.1817	93	0.9310	0.1044	0.9276
BMd01	40	0.1491	99	0.9253	0.7059	0.9208
BM200	58	0.1706	90	0.9213	0.1983	0.9166
BM188	49	0.1653	91	0.9191	0.8152	0.9140
PV_ctt001	23	0.1690	38	0.8860	0.0716	0.8752
BM141	38	0.2527	75	0.8740	0.2602	0.8642
BM175	39	0.2888	58	0.8707	0.0510	0.8616
BM183	41	0.2755	62	0.8695	0.1216	0.8589
BM172	47	0.3489	83	0.8580	0.1470	0.8527
BM160	59	0.3705	83	0.8497	0.0936	0.8460
BM205	19	0.3296	37	0.8219	0.2480	0.8035
BM139	27	0.4534	46	0.7679	0.0769	0.7564
BM201	16	0.3004	29	0.8152	0.0576	0.7920
BMd16	22	0.3096	35	0.7798	0.1743	0.7481
PV_ag003	12	0.3153	17	0.7685	0.0196	0.7329
BMd15	22	0.3476	33	0.7423	0.3111	0.6996
BMd18	15	0.3877	19	0.7111	0.3475	0.6663
BM149	10	0.5764	15	0.6328	0.0238	0.6086
PV_cct001	14	0.5000	17	0.6533	0.0724	0.6002
BMd08	14	0.5114	18	0.6716	0.0207	0.6345
BMd20	10	0.5397	13	0.6412	0.0146	0.5997
BMd47	10	0.4615	14	0.6554	0.0243	0.5952
BMd17	9	0.4892	12	0.6390	0.0784	0.5735
AG01	10	0.5780	17	0.5971	0.1951	0.5499
BMd02	12	0.5539	15	0.6012	0.0294	0.5420
PV_at003	14	0.4567	19	0.6056	0.1333	0.5258
BMd46	7	0.4930	10	0.5362	0.0099	0.4286
GATS54	9	0.6815	11	0.4555	0.0643	0.3811
BMd51	3	0.9894	3	0.0211	0.0000	0.0209

Only summary for 33 SSR markers shown

Chapter 5

Quality of core collections for effective utilization of genetic resources

Review, discussion and interpretation

ABSTRACT

Defining proper criteria for evaluating the quality of core collections is a prerequisite for selecting high-quality cores. However, a critical examination of the different methods used in literature for evaluating of the quality of core collections shows that there are no clear guidelines on the choices of quality evaluation criteria and as a result, inappropriate analyses are sometimes made leading to many false conclusions being drawn regarding the quality of core collections and the methods to select them. The choice of criteria for evaluating core collections appear to be based mainly on criteria being used in earlier publications rather than on the objectives of the core collection. In this study, an insight in the different criteria used for evaluating core collections is provided. We also discuss the different types of core collections and relate each type of core collection to possible evaluation criteria. Two new criteria based on genetic distance are introduced. The consequences of the different evaluation criteria are illustrated using simulated and experimental data. We strongly recommend the use of the distance-based criteria since they not only allow the simultaneous evaluation of all variables describing the accessions, but they also provide intuitive and interpretable criteria, as compared with the univariate criteria generally used for the evaluation of core collections. The results presented allow genebank curators and researchers to make informed choices when creating, comparing and using core collections.

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Quality of Core Collections for Effective Utilization of Genetic Resources
Review, Discussion and Interpretation

5.1 Introduction

Ex-situ germplasm collections have increased enormously in number and size over the last three to four decades as a result of global efforts to conserve plant genetic resources for food and agriculture. The large sizes of these collections complicate the characterization, evaluation, utilization and maintenance of the conserved germplasm. The approach of forming core collections (core sub-sets) was introduced to increase the efficiency of characterization and utilization of collections stored in the gene banks, while preserving as much as possible the genetic diversity of the entire collection (Frankel, 1984; Brown 1989). Frankel (1984) defined a core collection as a limited set of accessions representing, with minimum repetitiveness, the genetic diversity of a crop species and its wild relatives. From the original definition, several operational definitions have been coined (see Brown, 1995 and Van Hintum et al. 2000).

Core collections have many roles to play in the management and use of genetic resources. Gene bank curators have the responsibility for conservation, regeneration, safety duplication, documentation, evaluation and characterization of the genetic resources in their collections. These activities often require them to make choices or set priorities among accessions because of limited resources (Brown, 1995). Because a core collection is smaller in size compared to the whole collection, it enables some operations of the genebank, such as evaluation, to be handled more efficiently and effectively. The limited size of a core is key to its manageability, and in many cases the representation of the collection's diversity enables the core to function as a reference set of accessions for the whole collection (Brown and Spillane, 1999).

Since the inception of the idea of core collections over two decades ago, a body of literature on the theory and practice of core collections has accumulated. Very many approaches for selecting core collections have been proposed and used (*e.g.* M-Strat (Gouesnard et al. 2001), Genetic distance sampling (Jansen and van Hintum 2007), Power Core (Kim et al. 2007) and Core Hunter (Thachuk et al. 2009)). In comparing the options for assembling a core collection, one of the challenges is to decide on the evaluation criteria for the quality of the result. Various criteria for determining the suitability of a core collection have been suggested in the literature, yet very little attention has been given to the analysis of these quality criteria. In fact every researcher appears to have his/her own criteria for the evaluation of core collections.

There is a need to clearly define criteria for the evaluation of the quality of core collections and to determine the conditions under which these are suitable. For example, a core subset formed for the purpose of capturing rare or extreme traits (e.g. high resistance to pest or high yield) should be evaluated differently from one formed with the intention of representing an overview of the pattern of genetic diversity in the collection. By the pattern of genetic diversity we refer to the differences in the genetic constitutions of the accessions which have been accumulated as a result of natural processes, species characteristics and historical events.

In this paper, we will i) discuss the different types of core collections and proposed criteria suitable for quality evaluation of each type of core collection ii) discuss the different criteria used in the literature for evaluating the quality of core collections and relate each criterion to the different types of core collections iii) use real data sets (molecular marker data) to illustrate the performance of the proposed quality evaluation criteria with respect to the different types of core collections. The outcome of our study will allow researchers and curators to make informed choices from a set of alternative approaches.

5.2 What is a good core collection?

One of the key goals of defining a core collection is efficient utilization of available genetic resources and this is best achieved by having clear objectives in mind when selecting entries for the core (Mackay, 1995). The answer to the question “what is a good core collection” therefore depends on the objectives for making the core. This can be “storing as much variation as possible”, “optimizing the chance of finding a new allele” but also “obtaining a few accessions that represent the spectrum of phenotypes in the collection”. A second question is how to measure quality, and this will depend on the type of data available for evaluation.

According to Brown (1989), a good core collection should have no redundant entries (an entry is an accession included in the core), represent the whole collection with regards to species, subspecies and geographical regions and should be small enough to be easily managed. It was suggested by Marita et al. (2000) that selection of core collections can be performed with two general purposes i) maximizing the total genetic diversity(as sometimes favoured by taxonomists and geneticists) and ii) maximizing the representativeness of genetic diversity in the whole collection (as sometimes favoured by plant breeders). Accordingly, maximizing the representativeness of genetic diversity implies the inclusion of broadly adapted and heterotic materials containing 'generalist' alleles in a core collection. Earlier, Galwey (1995) stated these two purposes of core collections in a slightly different way as: (i) maximizing the representativeness of the full range of variation in whole collection; (ii) maximizing the representativeness of the pattern of variation in the whole collection.

There is also an aspect of balance between representing total diversity and the usefulness of the core to the intended user (Brown 1995). This can be illustrated with some examples. If a breeder searches for a particular trait, it is likely that the best core collection should contain relatively more material from the primary genepool as compared to the secondary genepool, irrespective of the amount of diversity in it, and within the primary genepool there will be a strong preference for material in an adapted genetic background. If a core collection is created in the search for new resistances, the part of the genepool that in the past has shown to contain resistances should obviously be overrepresented. This implies that the user is often not primarily interested in maximising diversity per sé (which would result in core collections with mainly wild and exotic material), but rather in optimising the chance of finding what he/she is looking for in material which is relatively easy to use in, say, a breeding programme. To achieve this, the selection of a core collection often starts with stratifying accessions into homogeneous groups, followed by an arbitrary determination of the number of accessions to be selected from each group, the so-called allocation. When a core collection is being formed for a specific user, the stratification and allocation process can be used to ensure that accessions from (a) particular group(s) (e.g. primary gene pool, modern varieties or Ethiopian landraces) are given more priority than justified by the genetic variation contained in that group. Since this stratification and especially the allocation process is sometimes arbitrarily defined by curators or users, it is difficult to incorporate this aspect into quality criteria. The rest of this paper will therefore concentrate on non-stratified groups of accessions. However, it should be noted that when grouping of accessions is necessary and/or when the groups can be appropriately determined, the quality criteria should be applied within the different groups.

From the literature, it is not clear how to relate the purpose of the core collections with the various quality evaluation criteria, and only very few authors have attempted this (e.g. Thachuk et al. 2009). Based on the purposes of core collections as suggested by Galwey (1995) and Marita et al. (2000), we have identified three broad types of core collection which will be discussed in the next section.

5.3 Types of Core Collections

Based on the purposes for which they are formed core collections can generally be classified into three categories. In defining the types of core collections, the term ***accessions*** refers to elements that constitute the whole collection (population) and ***entries*** are elements of the core collection (sample). Since the core collection is a selection from the whole collection, all entries are accessions, but only few accessions are entries.

Type 1: A core collection representing the *individual* accessions of the whole collection (CC-I).
Implication: each accession of the whole collection is represented by an entry of the core collection (usually by the closest entry).
This type of core collection (CC-I) aims at a uniform representation of the original genetic space, with equal weights across this space and is the most intuitive way of looking at core collection (see Fig 1). A core collection of type CC-I is especially suitable, for situations requiring an overview of the diversity of the accessions of the whole collection. Core collections formed for the purposes of maximizing the representativeness of genetic diversity as suggested by Marita et al. (2000) can be placed in type CC-I.

Type 2: A core collection representing the *extremes* of the whole collection (CC-X). *Implication*: the diversity of the traits of the entries of the core collection is maximized.
A core collection of type CC-X is geared towards representing the ranges of phenotypes or alleles of the whole collection. A good core collection of type CC-X has entries that are as different as possible from each other. A core collection representing the total genetic diversity, as suggested by Marita et al. (2000), can be considered as a core collection of type CC-X.

Type 3: A core collection representing the *distribution* of accessions of the whole collection (CC-D).
Implication: the distributions of all relevant traits with regard to the entries of the core are similar (in terms of mean, variance, quartiles, frequencies) to those of the whole collection.

This core collection of type CC-D is hardly ever of interest; only if the aim is to give an overview of a the composition of the whole collection using only a part of the collection, this type should be considered. This type of core collection will be obtained by maximizing the representativeness of the pattern of variation of traits in the whole collection, as suggested by Galwey (1995).
Although a CC-D core collection is hardly of interest, the criteria used for evaluating most core collections in the literature suggest that most core collections are of type CC-D (*e.g.* annual medicago (Diwan et al. 1994), sesame core collection - China (Xiurong et al. 1998), Iberia Peninsula common bean (Rodino et al. 2003), groundnut (Upadhyaya et al. 2003), peanut (Valencia) (Dwivedi et al. 2008), USDA soybean core (Oliveira, et al. 2010)).
The different types of core collections have been illustrated graphically using a multimodal univariate distribution for the whole collection (Fig 1).

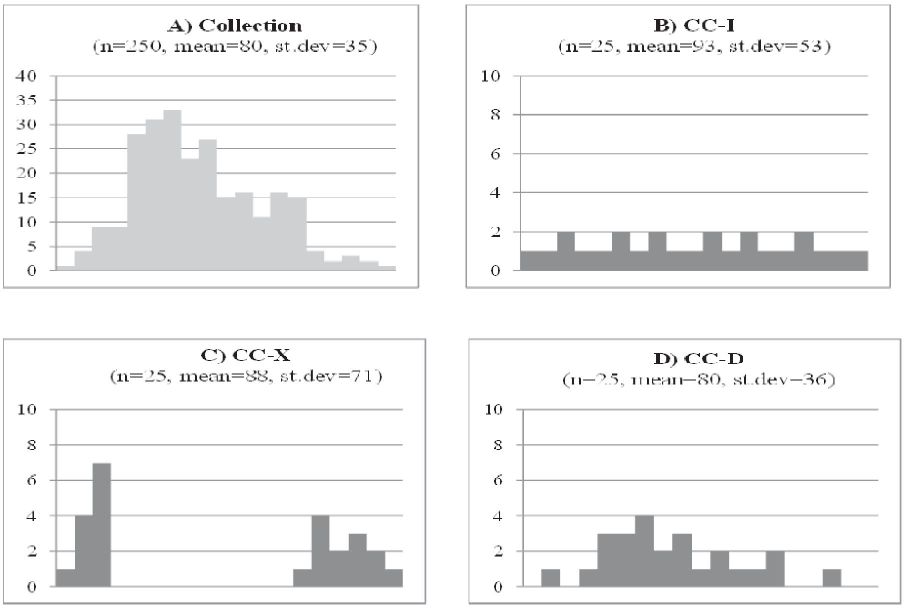

Fig 1: A. Multimodal trait distribution of for whole collection; B. Distribution of the same trait for a collection of type CC-I; C. Distribution of the same trait for a core collection of type CC-X ; D. Distribution of the same distribution for a collection of type CC-D

5.4 Quality criteria for evaluating core collections

The process of evaluating a core collection usually involves a comparison with the whole collection from which it has been obtained, or a comparison with alternative core collections. This requires clear and objective criteria for assessing the quality of the different types of core collections.

Irrespective of the type of core collection and the quality criterion used, the evaluation of quality should be based if possible on data that were not used in the selection of the core (van Hintum et al. 2000). This might sound like an obvious statement, but it is very often neglected (e.g. Tai and Miller 2001 and Wang et al. 2007). For example, one has a dataset of 1000 accessions each genotyped with 50 markers, and the objective is to create a core collection of 20 entries with maximal allelic richness. If it would concern only the current 50 markers, this would be a simple optimisation problem. However, the question is, “what if the core collection should also be ‘allelic rich’ for all loci that were not genotyped?” One option would be to use half the markers for creating core collections using different methods, and the other half for evaluating the quality of the result of each method (for a good examples see Mckhann et al. (2004), Ronfort et al. (2006) and Balfourier et al. (2007)). Once the best strategy has been determined this strategy could then be used on the entire set of markers to create the final core collection. Since often molecular data will be used to select a core that is also supposed to optimize the phenotypic diversity, relevant phenotypic traits should be used for the validation.

In this article, we place emphasis on evaluation criteria based on genetic distances between accessions. The main advantage of using genetic distance for evaluation of core collections is that unlike the other criteria used in literature which handle one variable at a time, all the variables are used simultaneously. It is also easier and more intuitive to link distances to the concept of genetic diversity.

Evaluation of type CC-I

A good criterion for evaluation CC-I core should relate each accession of the whole collection with the entries of the core collection. For CC-I, we proposed criteria based on distances between each accession in the whole collection and the nearest entry in the core collection (A-NE) (see Fig 2).

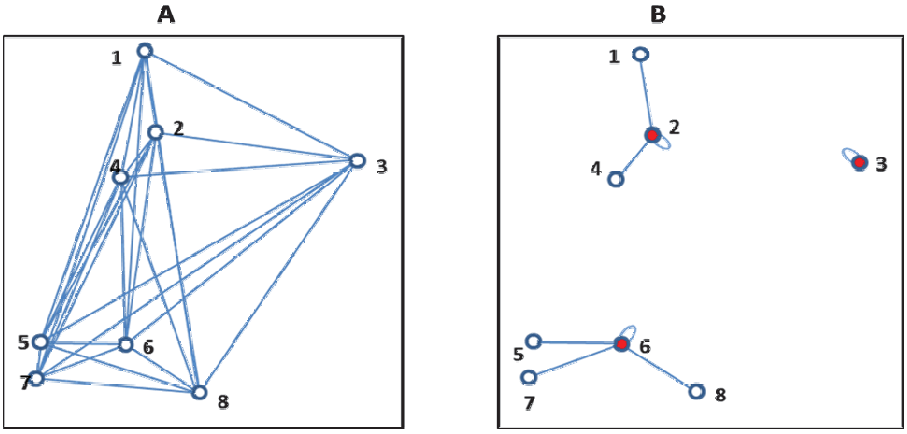

Fig 2: A) Eight accessions (1,2,..,8) in a 2D space with all pairwise distances (the distance between accession ***n*** and ***m*** is indicated as $\boldsymbol{D_{n-m}}$). B) The three selected entries (highlighted accessions) based on the A-NE criterion, minimising the average distance between each accession and it nearest neighbouring entry ($D_{1-2} + D_{2-2} + D_{3-3}$+ $D_{4-2} + D_{5-6} + D_{6-6} + D_{7-6} + D_{8-6}$)/8

Average distance between each accession and the nearest entry (A-NE) (Odong et al 2011b): In this case, the distance between each accession and the nearest entry in the core is calculated and averaged over all the accessions. For the selected accessions (entries) these distances are taken as zero (they are closest to themselves). For example, the value A-NE for Fig 2 is given as

$$A - NE = \frac{(D_{1-2} + D_{2-2} + D_{3-3} + D_{4-2} + D_{5-6} + D_{6-6} + D_{7-6} + D_{8-6})}{8}$$

Where the distance between accession ***n*** and ***m*** is indicated as $\boldsymbol{D_{n-m}}$.

For core collections of type CC-I, the value of A-NE should be as small as possible; the maximum representation (A-NE = 0) is obtained when each accession is represented by itself or by an identical duplicate accession in the core. In core collections that optimize the values of A-NE (CC-I type of core), the accessions selected as entries tend to be centres of clusters(groups) rather peripheral objects (see Fig 4).

Evaluation of type CC-X

A good criterion for a core collection of type CC-X (representing the extreme values) should be able to quantify differences between entries of the core collection as well as being able to measure the inclusion or exclusion of accessions with extreme traits in the core. The most intuitive criteria for determining differences between entries in the core collection are those criteria based on pair-wise distances. The exclusion or inclusion of accessions with extremes values in the core can be assessed using frequencies of traits or alleles captured (see Thachuk et al. 2009). Below we propose a new criterion based on distances between an entry and the nearest neighbouring entry (E-NE) and compare it with criteria based on average pair wise distances between all entries.

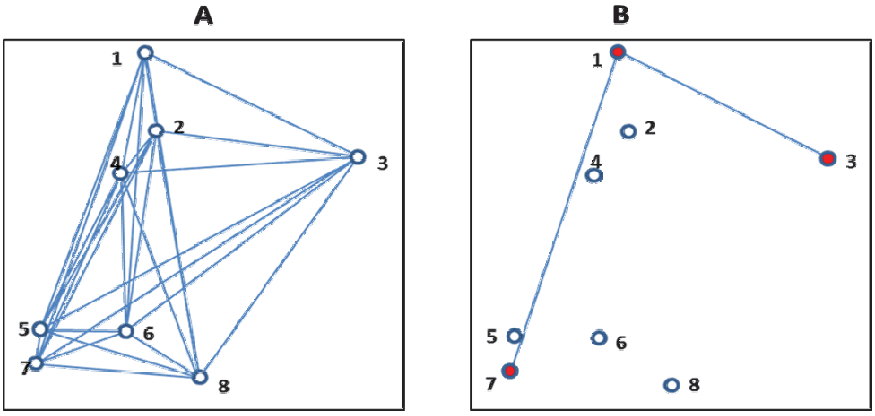

Fig 3: A) Eight accessions (1,2,..,8) in a 2D space with all pairwise distances (the distance between accession ***n*** and ***m*** is indicated as $\boldsymbol{D_{n\text{-}m}}$). B) The three selected entries (highlighted accessions) based on the E-NE criterion maximizing distances between each entry and the nearest neighbouring ($D_{1\text{-}3}$ + $D_{3\text{-}1}$ + $D_{7\text{-}1}$)/3

***Average distance between each entry and the nearest neighbouring entry (E-NE)*:** According to this criterion (E-NE), a good core collection is one that maximizes the average distance between each entry and the nearest neighbouring entry in the core collection. For this criterion, each entry should be as different as possible from the most similar entry. This avoids selecting a few clusters of similar accessions at the extreme ends of the distribution, that might occur if one chooses a set of entries that maximizes the average of all pair-wise distances between the entries in the core (E-E) (see Fig 4). Using example in Fig 3, if accessions 1,3 and 7 are selected as entries in the core collection, and if 1 is the nearest neighbouring entry to both 3 and 7 (reverse is also true) then E-NE is given as

$$E - NE = \frac{(D_{1-3} + D_{3-1} + D_{7-1})}{3}$$

where the distance between accession ***n*** and ***m*** is indicated as $\boldsymbol{D_{n\text{-}m}}$.

Average genetic distances between entries (E-E): Maximizing the average genetic distance between entries of a core collection has been suggested as a desired quality criterion for evaluating core collections intended for plant breeders (Franco, 2006, Thachuk et al. 2009). Using example in Fig 3, E-E are given as

$$E - E = \frac{(D_{1-3} + D_{1-7} + D_{3-7})}{3} \quad .$$

Fig 4 provides a simple numeric and graphical comparisons of the three distance-based criteria discussed above. Although both E-E and E-NE are suitable for CC-X type of core, as illustrated in Fig 4C core collection with a high average distance between the entries (E-E) can still have a high level of redundancies. It is clear from Fig 4 that despite having the highest E-E (0.573 versus 0.491 and 0.467) the core collection in Fig 4C, some entries in Fig 4C are too close to each other to be included in a core collection as reflected by a low value of E-NE. Fig 4 A indicates that minimization of A-NE leads to selection of accessions from the centres of clusters compared to E-E and E-NE which select accession at the periphery of clusters.

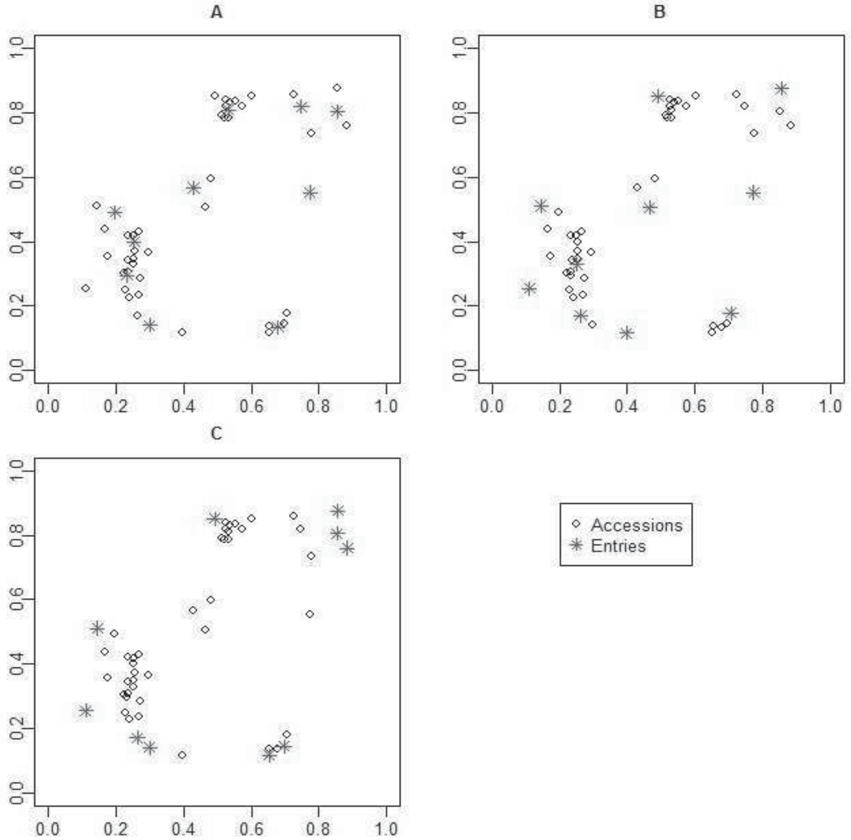

Fig 4 Examples of core collections, showing the effect of optimization of different criteria on the positioning of entries (red stars) within the distribution of accessions (circle) for each core collection, the value of all three evaluation criteria are given: A) The average distance between each accession and the nearest entry (A-NE) is minimized (E-E =0.467; E-NE=0.180; A-NE=0.038) B) The average distance between an entry and the nearest other entry (E-NE) is maximized (E-E =0.491; E-NE=0.241; A-NE =0.056) C)The average distance between entries (E-E) is maximized (E-E =0.573; E-NE=0.118; A-NE=0.094). Thus, for E-E and E-NE, the larger the value the higher the quality of the core collection, the opposite is true for A-NE.

Evaluation of type CC-D

Ideal criteria for evaluating a core collection of type CC-D should be able to compare many distributional aspects simultaneously: centre (mean, mode), spread (variance, range), shape (symmetry, skewness, number of modes) and unusual features (gaps, presence of outliers) of all data simultaneously. For continuous data, we propose the use of quantile-quantile plots (Gnanadesikan and Wilks 1968) which provide a visual comparison for two data sets using several distributional aspects of the data simultaneously. We also recommend the use of Kullback-Leibler distance (Kullback and Leibler, 1951) which measures the distance between probability distributions, can be used to compare the difference in probability distribution between the core collection and the whole collection.

QQ plot: Compared to simple comparison of means or variances the QQ plot gives a much better overall visual view of how the distribution of a given trait differs between the core collection and the whole collection. A QQ plot is a graphical method for comparing two probability distributions by plotting corresponding quantiles against each other. If the two distributions are similar, the points in the QQ plot will lie approximately on a straight line. A QQ plot is generally a more powerful approach for comparing distributions than the common technique of comparing histograms of the two samples, but requires more skill of interpretation. A more quantitative approach for comparing the distribution of the traits in the whole collection and the core would be to calculate the Kullback-Leibler distance between the core collection and the whole collection. Fig 5 below shows QQ plots for the three core collections shown in Fig 1. We have also used the information from QQ plot to calculate the Kullback-Leibler distance between the different core collections in Fig 1 and the whole collection. A brief description of Kullback-Leibler distance is presented in Appendix 1.

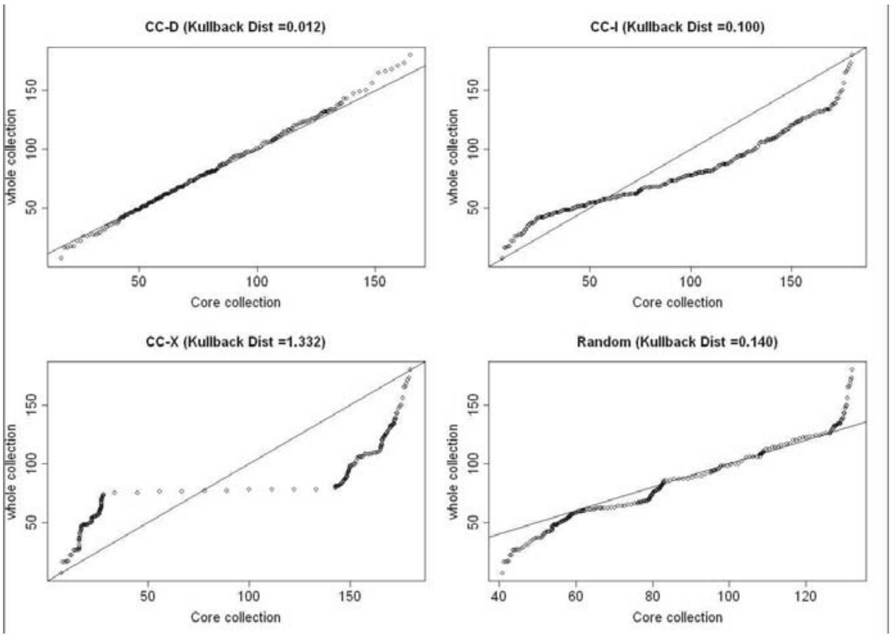

Fig 5: Q-Q-plots for different types of core collections shown in Fig 1. From both the qq-plots and Kullback distance (Kullback Dist), it is clear that the distribution of whole collection is best represented by type 1 (CC-D) core. The Kullback-Leibler distance was calculated based on values generated by the Q-Q plot. Random sampling core collection is only based on one data set. The minimum value of Kullback-Leibler distance is zero (for a core collection with identical distribution to that of the whole collection).

5.5 Common methods used for evaluating core collections in the literature

Below we give an overview of the various criteria for evaluating core collections used in the literature and relate them to the three types of core collection. Given that the type of data determines how diversity in the whole collection or the core collection should be quantified, we will also try to relate the evaluation criteria to the different types of data (see Appendix 2 for brief descriptions of different types of data used for selecting and evaluating the quality of core collections). It should be noted that when evaluating the quality of core collections, most authors apply several evaluation criteria despite the fact that those criteria are only suitable for different aspects of core collections. The most common criteria used for evaluating core collections include summary statistics, the Shannon diversity index, class/category coverage and chi-square tests of association (see table 1 below for summary).

Table 1: Summary of common criteria used for evaluation of the quality of core collections in literature

Criteria	**Type of variables**	General comments[1]
Summary statistics	Continuous	- Compare the mean, variance, etc. of the core with that of whole collection - Comparison is done for one variable at a time and later combined - Mainly suitable for CC-D type of core collections
Principal component analysis	Continuous	- Plot of the coordinates of the entries on the main principal components (exploratory) to show spatial distribution of entries and accessions - Compare two core collections using sum of squares of the their scores along the major PCs (Suitable for CC-X core type)
Shannon diversity Index (SH)[2]	Categorical	- The highest value is obtained when all the categories in the whole collection are represented in equal proportion (penalizes redundancy at the category level). - The value of SH of a given core collection should be compared with the maximum possible value (***log (n)***, where n is the number of classes in the whole collection) - Most authors apply this criterion inappropriately by comparing SH value of the core collection with that of the whole collection. - Suitable for CC-I core type
Class coverage[2]	Categorical	- The highest value (1 or 100%) is obtained when all the categories in the whole collection represented in the core - Unlike SH it does not correct for redundancy in the core collection - Suitable for CC-I core type
Chi-square goodness-of-fit[2]	Categorical	- This criterion has been used to test for the deviation of the frequency distributions of important categorical traits between core collection and the whole collection - A good core collection is one in which the frequency distribution of the categories of the core is not statistically different from that of the whole collection - Suitable for CC-D core type

[1] For all criteria except Principal component, the criterion is calculated for each variable at a time and later combined

[2] Can be applied to ccontinuous variables by first putting values into specific number of classes (determining the number classes is challenging)

5.5.1 Summary statistics: Criteria based on mean, variance and other summary statistics such as coefficient of variation, range, inter-quartile range have been used mainly to evaluate the quality of core collections based on continuous traits (Hu and Xu 2000; Tai and Miller, 2000). It involves statistical tests of differences between means, variances and other summary statistics of the core and the whole collection. Based on the results of statistical tests (mainly t-tests and F-tests) performed on each trait separately, several evaluation criteria (mean difference percentage, variance difference percentage, coincidence rate of change and variable rate of coefficient of variation, sign test) have been suggested (see Table 2). Criteria based on means and variances are probably suitable for the evaluation of a core collection of type CC-D and will perform very poorly with core collections of type CC-I and CC-X.

Some authors have questioned the use of differences between means and variances of core and whole collection as criteria for evaluating the quality of core collections (e.g. Kim et al. 2007). There is also a conceptual problem when comparing a core collection (a sample) and a whole collection (population). Thus the question is not whether these two samples are different, but could this sample have come from this distribution? So we should be dealing with a one-sample test and not a two-sample test. It is thus clear that the use of QQ plot (Gnanadesikan and Wilks 1968) and probability distribution based methods such as the Kullback-Leibler distance (Kullback and Leibler 1951) would be the best option for evaluation of CC-D types of core collections.

Table 2: Common criteria for evaluating the quality of core collections based on summary statistics

Criteria	**Description**
Mean difference percentage (MD) (Hu et al. 2000[3])	$$MD = \left(\frac{S_t}{n}\right)x100$$ where S_t is the number of traits with a significant difference between the means of the whole collection and the core collection; n is the total number of traits. The lower (<20%) the value of MD the more representative the core collection.
Variance difference percentage (VD) (Hu et al. 2000)	$$VD = \left(\frac{S_t}{n}\right)x100$$ where S_t is the number of traits with a significant difference between the variances of the whole collection and the core collection; n is the total number of traits. The larger (>80%) the value of VD, the more diverse the core collection.
Coincidence rate of range (CR) (Diwan 1995)	$$CR = \frac{1}{n}\sum_{i=1}^{n}\frac{R_{C(i)}}{R_{W(i)}}x100$$ where $R_{C(i)}$ and $R_{W(i)}$ represent the ranges of the i^{th} trait in the core collection and the whole collection, respectively; n is the total number of traits.
Variable rate of coefficient of variation (VR) (Hu et al. 2000)	$$VR = \frac{1}{n}\sum_{i=1}^{n}\frac{CV_{C(i)}}{CV_{W(i)}}x100,$$ where $CV_{C(i)}$ and $CV_{W(i)}$ represent the coefficients of variation of the i^{th} trait in the core collection and the whole collection, respectively; n is the total number of traits.
The Sign test (Basigalup et al. 1995, Tai and Miller, 2001)	$$X^2 = (N_1 - N_2)^2 / (N_1 + N_2).$$ where N_1 is the number of variables for which the mean or variance of the core core collection is greater than the mean or variance of the whole collection (plus); N_2 is the number of variables for which the mean or variance of the core collection is less than the mean or variance of the whole collection (minus). The values of X^2 should be compared with a chi-square distribution with 1 degree of freedom.

[3] For a core collection to be representative of the whole collection, the value of MD should not be more than 20% and the value of CR should be greater than 80% (Hu et al 2000).

Apart from the criteria described in Table 2, the correlation coefficient has also been used as a criterion for evaluating the quality of core collections (Reddy et al. 2005; Mahajan et al. 2007). The pairwise phenotypic correlation coefficients between different traits are calculated separately for the core collection and whole collection and the values are then compared in order to determine whether the associations between traits have between conserved well enough in the core collection.

5.5.2 Principal component analysis

Another exploratory criterion for evaluating core collections involves the inspection of the spatial distribution of the entries in plots of principal components (Bisht, Mahajan and Patel, 1998; Kang et al. 2006, Mahajan et al. 2007). Based on the method suggested by Noirot et al. (1996), it is possible to compare two core collections or relate the core collection with the whole collection based on the sum of squares of the scores of the entries on the major principal components: the greater the value, the more diverse the core collection. This criterion would be suitable for evaluation of core collections of type CC-X. However, it should be noted that a core with a higher value for this criterion can still have a high level of redundancy resulting from the inclusion of two or more similar accessions from the extreme ends of the distribution.

5.5.3 Shannon diversity Index (SH):

This criterion is suitable for evaluating core collections using categorical data; it has been used extensively in the literature. For a given trait, the Shannon diversity index (Shannon, 1948) is calculated as follows:

$$SH = -\sum_{i=1}^{n} p_i \log(p_i)$$

where p_i is the frequency of the category i and n is the total number of categories. The SH penalizes redundancy at the category level and its maximum value (***log(n)***) is obtained when all classes are represented in equal proportions ($i.e.\ p_1 = p_2 = \ldots = p_n = 1/n$). Therefore, in terms of SH, the best core collection should be the one with the maximum attainable value which makes SH a suitable criterion for core collections of type CC-I. It is completely meaningless to make comparisons of SH values of the core collection and the whole collection as often done in the literature (*e.g*. Bisht, Mahajan and Patel (1998), Upadhyaya et al. (2003), Mahalakshmi et al. (2007), Dwivedi et al. (2008) and Upadhyaya et al. (2009)) since SH of the whole of collection is often affected by high level of redundancy which we would not want to have in the core collection.

To apply SH or other measures of diversity to continuous agronomic data, the data should first be converted into categorical data by putting them into a specific number of classes. McKhann et al. (2004) suggested that instead of calculating SH for each trait separately, traits (any type of traits) should be used to calculate distances between each accession and the centre of the distribution represented by multi-trait mean values after which, the observed range of the distances is divided into several discrete classes of equal length. One of the main problem with this approach is that two accessions with equal distances from the centre of the distribution but on the opposite sides are put in the same category.

5.5.4 Class Coverage (Coverage):

This reports the percentage or proportion of the categories in the whole collection that have been retained in a core collection (Kim et al. 2007). It is defined by

$$Coverage = \left(\frac{1}{K}\sum_{k=1}^{K}\frac{A_{Core}}{A_{Wcol}}\right)x100$$

where A_{Core} is the sets of categories in the core collection and A_{Wcol} is the sets of classes found in the whole collection and K is the number of traits. According to this criterion, a good core collection should retain all categories of a given variable in the whole collection. For the case of molecular marker data, the categories represent the number of distinct alleles (akin to allelic richness) in the whole collection. Class coverage is also a suitable quality criterion for core collections formed with the purpose of representing the accessions in the whole collection (type

CC-I) and when applied to molecular markers it will be suitable for core collections aimed at capturing accessions with rare alleles (type CC-X).

It should be noted that unlike SH, *coverage* does not take into consideration the differences in frequency of the categories represented in the core collection so a core collection with high *coverage* can still have high redundancy. Just like with SH, deciding on the number of categories (intervals for continuous data) is a major challenge when calculating *coverage*.

5.5.5 Chi-square goodness-of-fit:

This criterion has been used to test for the deviation of the frequency distributions of important categorical traits between core collection and the whole collection (Tai and Miller 2001, Grenier et al. 2000, Zeuli and Qualset 1993). Chi-square goodness-of-fit can also be used for continuous agronomic data converted into categorical data. The chi-square values can be computed as:

$$\chi^2 = \sum_{i=1}^{k} \frac{(CFreq_i - WCFreq_i)^2}{(WCFreq_i)}$$

where $CFreq_i$ is the relative frequency of accession from category i ($i = 1,2,....,k$) in the core collection and $WCFreq_i$ is the relative frequency of accessions from category i in the whole collection. The number of degrees of freedom being the number categories (classes) minus one. This test (chi-square) is only suitable when the interest is in representing the distribution of accessions in the whole collection (type CC-D).

From the literature, it clear that criteria based on summary statistics and SH are the most frequently used (see Table 3). Because of the similarities of criteria used to evaluate those core collections, it appears that all those cores were obtained with the same objective(s) in mind.

Table 3: Samples of Core collections from literature showing data and criteria used for their evaluating them

Paper (Core)	Data use for selection	Data use for evaluation	Criteria use for evaluation
Soybean core collection (Oliveria et al. 2010)	P, A ,M	A, M	Summary statistics, chi-square, Correlations
Sorghum mini core (Upadhyaya et al. 2009)	P, A, M	P, A ,M	Summary Statistics, Chi-square, SH, Correlation
Mini core Japanese rice landraces (Ebana et al. 2008)	Markers	Markers, A	Percentage of alleles retained, Summary Statistics
Peanut (Valencia) (Dwivedi et al. 2008)	P, A, M	P, A, M	Summary statistics, Chi-square SH Correlation
A worldwide bread wheat (Balfourier et al. 2007)	P, Markers	P, Markers*	Alleles captured , countries of origins represented
Pearl millet (Bhattacharjee et al. 2007)	P, A, M	P, A, M	Summary Statistics, Chi-square, SH, Correlation
World sesame (Mahajan et al. 2007)	P, A, M	A, M	Summary statistics, Correlations, SH, PCA
West African yam Dioscorea spp. (Mahalakshmi, et al. 2007)	P, A, M	A	Summary Statistics , Correlation Chi-square, SH
USDA rice (Yan et al. 2007)	P	A, M*	Summary Statistics, Correlation
Korean Sesame core (Kang et al. 2006)	P, A, M	A, M	Summary Statistics, Chi-square PCA
Pigeon pea (Reddy et al. 2005)	P, A, M	P, A, M	Summary Statistics, Chi-square, SH, Correlation
Iberia Penisula common beans (Rodino et al. 2003)	P	A, M	Summary statistics, Chi-square
Groundnuts (Upadhyaya et al. 2003)	P, M	M	Summary statistics, chi-square, SH, Correlation
Sesame -China (Xiurong, et al. 2000)	P, A, M	A, M	Summary Statistics
Indian Mung Beans (Bisht, Mahajan and Patel, 1998)	A, M	M*	Summary Statistics, PC, SH
Perennial *Medicago* (Basigalup et al. 1995)	P , A, M	A, M	Summary Statistics
Annual Medicago (Diwan et al. 1994)	P, A, M	P, A, M*	Summary Statistics

A*: Agronomic data,* **M***: Morphological data,* **P***: Passport data,* **PCA***: Principle component analysis,* **SH:** *Shannon Diversity Index*
**Part or all the data used for evaluation was different from the one used for forming the core collection*

5.6 Illustration using real data sets

We used two published data sets (Coconut and Common bean (Odong et al. 2011)) to show the importance of choosing the right criteria for each type of core collection (see chapters 2 and 4 for detail description of the data). Core collections of different sizes (5 to 100) were formed by optimizing (minimizing or maximizing) each of the three criteria (A-NE, E-NE and E-E) and later evaluated using the other two criteria.

For both coconut and common bean data, Fig 6 shows that in terms of A-NE (representing accessions in the whole collection), core collections formed by maximization of E-NE or E-E perform even poorer than random sampling. On the other hand the performance of core collections formed by minimizing A-NE performed poorly when evaluated using E-NE or E-E criteria (see Fig 7 and 8). This shows that when selecting a core collection, it is essential to define the objectives clearly and the objectives should be the basis for choosing the evaluation criteria.

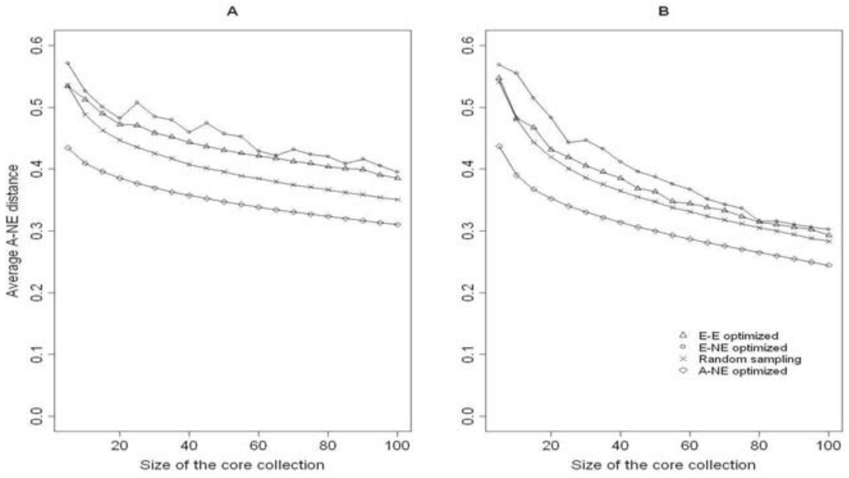

Fig 6: Plot of Average distance between each accessions and its nearest entry in the core (A-NE) against different sizes of collections formed by optimizing (minimizing or maximizing) different criteria (E-E, E-NE, A-NE and Random sampling) using Coconut (A) and Common beans (B).

We have shown in Fig 7 and Fig 8 that for both crops a core collection that maximizes E-NE also perform (maximizes) very well with respect to E-E but the reverse is not always true (*i.e.* maximizing E-E can results in a much lower value of E-NE since similar accessions at the extreme ends of the distributions can be included in the core). In general, for both coconut and common beans data sets comparison based on E-E is less responsive to changes within the core collection introduced by either changing the number entries (5 - 100) or changes in the optimization methods used for forming core collection. For example for both crops (Fig 7 and 8) the changes in E-E between a core of size 5 and that of size 100 range between 1.5 to 12% compared to the changes in E-NE which lies between 18 to 54%. The little response of E-E to changes within core collection is due to the fact that as the core (sample) size increases, the average distance between entries (E-E) tends towards the overall mean of distances between accessions in the whole collection (the E-E line of random sampling – Fig 7 A and Fig 8A).

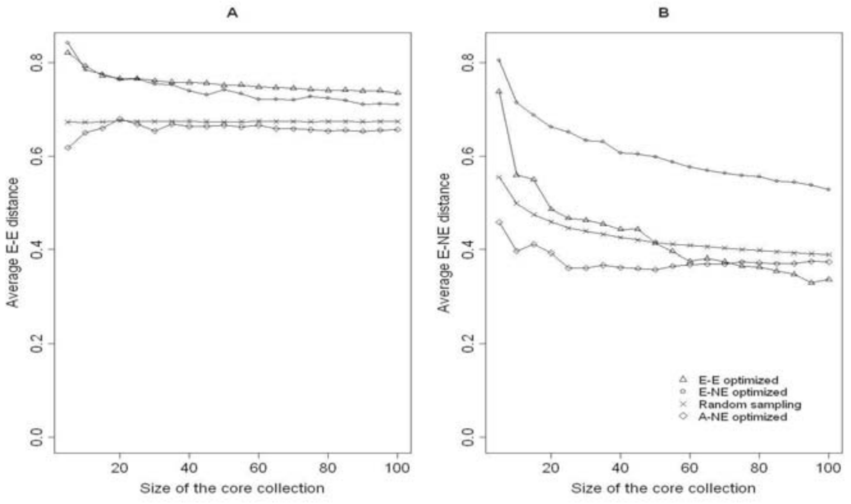

Fig 7: Plot of average distances between the entries in the core collection (E-E) (A) and average distance between an entry and the nearest neighbouring entry (E-NE) (B) against the size of core collection for cores formed by optimizing different criteria (E-E, E-NE, A-NE and random sampling) for Coconut data (1014 accessions)

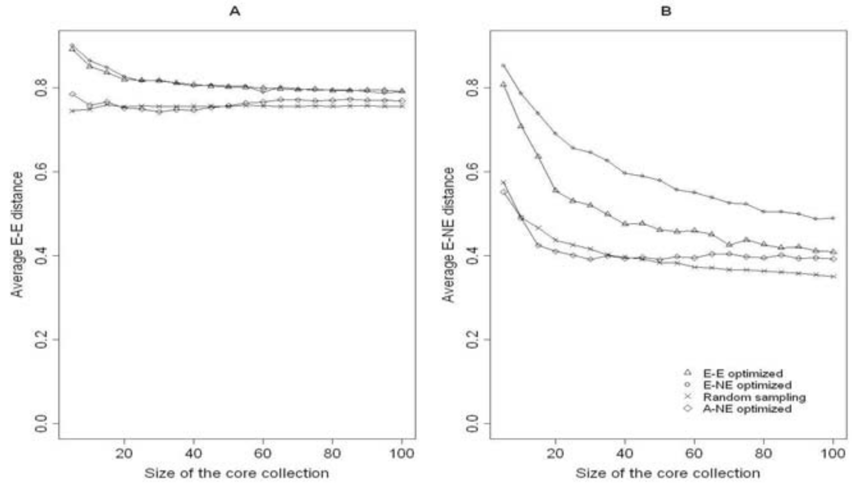

Fig 8: Plot of average distances between the entries in the core collection (E-E) (A) and average distance between an entry and the nearest neighbouring entry (E-NE) (B) against the size of core collection for cores formed by optimizing different criteria (E-E, E-NE, A-NE and random sampling) for Common bean (515 accessions) data

Use of different data sets for evaluating core collections

A core collection obtained by optimizing one set of variables may not be optimal for another set of variables. The evaluation of a core collection with the same data set that was used to create it ignores this simple but very important point. This is quite important especially in the case of molecular markers data where the key assumption is that by maximizing diversity in a given set of markers loci, the diversity at genes of interest will also be maximized. Fig 9 shows the result obtained by dividing the common bean data into two sets; one set (random sample of 18 SSR markers) was used to form the core collections (*training set*) and the other set (the remaining 18 SSR markers) was used to evaluate the resulting cores (*evaluation set*). It is clear from Fig 9 that major differences may occur between the unknown value we intend to optimize (Target – obtained by optimizing evaluation set) and the actual value obtained when the core is formed using training set and evaluated using another set of data (Actual – obtained by optimizing training set and evaluated using evaluation set). Although the core collections obtained by optimizing both E-NE and A-NE performed better than random sampling in capturing unknown diversity, the differences are quite small (5 -15% for E-NE and 1-5% for A-NE). Ronfort et al. (2006) found very little gain in the total number of alleles captured using the H and M strategy (Schoen and Brown, 1995) over random sampling when evaluation was done using a different set of data. Their (Ronfort et al. 2006) major explanation was that the set of inbred lines used in their study had no redundancy leaving little room for optimization to improve the results over and above random sampling. The relatively small gain in our case is probably due to limited size (number of markers) and questionable quality of the data. For data set with limited structure, we expect little gain by minimizing A-NE compared to random sampling and this could explain the small difference observed in the common bean data *i.e.* splitting the common bean data into two weakened the group structure in data resulting into very little gain.

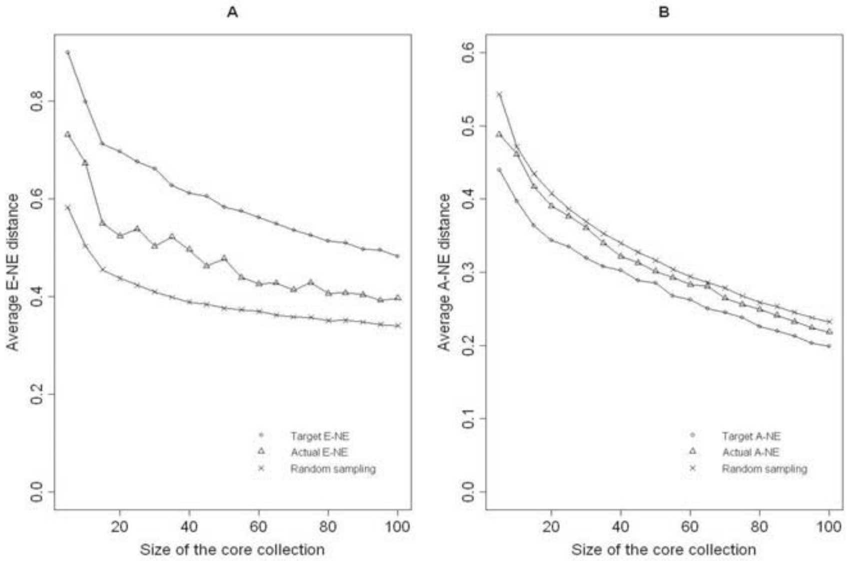

Fig 9: Plot of average distance between an entry and the nearest neighbouring entry (E-NE) (A) and average distance between each accessions and its nearest entry in the core (A-NE) (B) against the size of core collection for bean data set. The bean data set was split into two halves with one half used to form collection and the other half used for evaluation of the core. Target (E-NE and A-NE) values are the maximum (E-ENE) or minimum (A-NE) possible values for each criterion for the half of the data used for evaluation (*evaluation set*), while actual (E-ENE and A-NE) values are obtained from a core collections that were created using one half (training set) and evaluated using the quality evaluation half of the data (evaluation set).

5.7 Conclusions and recommendations

A critical examination of the different methods for evaluating the quality of core collections used in the literature shows that the choices of criteria for evaluating core collections are sometimes meant arbitrarily resulting in false conclusions regarding the quality of core collections and the methods to select them. The criterion of choice for evaluating the quality of core collections should be determined by the objectives or type of the core collection. If the core collection is made to represent the accessions in the collection (CC-I), the evaluation criterion should reflect that, and a criterion such as the A-NE criterion proposed in this paper should be used. If the core is to represent the range of genotypes and/or phenotypes in the collection (CC-X), a criterion such as the E-NE criterion should be used. In addition, we stress that where possible or appropriate the evaluation of core collections should be based on data that are not used for the selecting the core collection. When the core collection is intended for a specific user, the quality will have to be determined in terms of fitness-for-use such as the ease with which certain groups of material can be used or the likelihood of finding traits of interest.

In summary, we introduced two distance-based criteria (A-NE and E-NE) for evaluating the quality of core collections. We strongly recommend distance-based criteria mainly for two reasons a) they combine information from all traits simultaneously, instead of using one trait at a time as most of the evaluation criteria used in literature do, b) they are intuitive, easy to interpret and relate to the concept of representation of genetic diversity. The new distance based criteria, we proposed in this paper, are suitable for evaluating the two important types of core collections (CC-I and CC-X) These evaluation criteria can also be used as optimisation criteria when creating the core collections.

Appendices

Appendix 1: brief description of types of data used for selection of core collections

Several types of information can be used for selecting core collections. The most common type of data used include i) passport data ii) agronomic data and iii) molecular marker data.

Passport data

Passport data are data about the identity and origin of the accession, including its taxonomic classification, with connected knowledge about domestication, distribution, breeding history, cropping pattern and utilization. Example of passport data include the country of origin, the crop type (e.g. winter versus summer wheat), and pedigree.

Agronomic data

Agronomic data can be continuous, discrete or categorical. Examples of continuous variables include grain yield, plant height, leaf area, etc. Discrete variables mainly deal with counts such as the number of fruits or the number of seeds in a pod. Categorical variables may be defined as binary (presence or absence of a given characteristic), nominal (colour or shape of an organ) or ordinal (a visual scale arranged to represent intensity, color or size) (Crossa and Franco 2004). Agronomic traits are usually controlled by multiple genes as well as by environmental factors.

Molecular data

Data from molecular or biochemical marker systems can be treated as either continuous (allele frequency) or categorical (presence or absence of band or allele). Examples of popular molecular data types include single nucleotide polymorphism (SNP), amplified fragment polymorphism (AFLP), random amplified polymorphic DNA (RAPD), and simple sequence repeats (SSR).

Appendix 2: Description of Kullback-Liebler distance

In probability theory and information theory, the Kullback–Leibler distance (KL) is a non-symmetric measure of the difference between two probability distributions *P* and *Q* (Kullback and Leiber, 1951). For two probability distributions P and Q, KL distance is defined as

$$K(P,Q) = E_p\left[\log\left(\frac{Q}{P}\right)\right]$$

where $E_p[.]$ indicates the expectation value with respect to the probability distribution *P*(the expectation is evaluated with respect to distribution *P*).

Typically *P* represents the "true" distribution of data, observations, or a precise calculated theoretical distribution. The measure *Q* typically represents a theory, model, description, or approximation of *P*. KL is always non negative and is zero only if the two distributions are identical. In core collection application, the distribution of a particular trait in the whole collection represents true distribution (P) which is approximated by the distribution of the trait in the core collection. KL distance would therefore be a suitable criterion for evaluating core collections selected for representing the distribution of the traits in the whole collection.

For normally distributed variables, KL distance can be calculated for univariate as well as multivariate data. For two multivariate normal densities KL is an explicit function of only their covariance (correlation) matrices (Σ_1 and Σ_2) and the only necessary condition is that the two covariance matrices be positive definite (Tumminello et al. 2007, Chen et al. 2008). Given two probability density functions $P(\Sigma_1, X)$ and $P(\Sigma_2, X)$ KL is defined as

$$K(P(\Sigma_1, X), P(\Sigma_2, X)) = \frac{1}{2}\left[\log\left(\frac{|\Sigma_2|}{|\Sigma_1|}\right) + tr\left(\Sigma_2^{-1}\Sigma_1\right) - n\right],$$

where n is the dimension of the space spanned by the X variable and $|\Sigma|$ indicates the determinant of Σ.

There are several other distance (probability) based criteria that can be used to compare the two distribution (example: Kolmogorov-Sminov test; Anderson-Darling distance (see Stephens, 1977)).

Chapter 6

General discussion

6.1 Introduction

Since its inception about three decades ago, the core collection concept has been fully accepted, and made operational in many genebanks around the world (see Huaman et al. (1999), Malosetti and Abadie (2001), Upadhyaya et al. (2001), Li et al. (2002), Wang et al. (2006), Balfourier et al. (2007) and Mario et al. (2010)). In this thesis efforts have been made to combine existing knowledge on core collections and statistical-genetic concepts to aid the efficient and effective utilization of genetic resources. The research was aimed at filling knowledge gaps in the development and utilization of core collections. In this final chapter, the main findings of the thesis and their implications will be discussed and suggestions will be made for future research.

In this thesis three key aspects of core collection development and plant genetic resources utilization have been considered:

a) methods for the determination of the genetic structure of germplasm collections and the relevance of genetic structure for the selection of core collections and the utilization of germplasm resources (Chapters 2 and 3)

b) methods for connecting germplasm collections stored in different genebanks around the world using molecular marker data (Chapter 4) and

c) a critical examination of criteria for evaluating the quality of core collections (Chapter 5).

6.2 Methods for the determination of the genetic structure of germplasm collections and the relevance of genetic structure for genetic resource utilization

Understanding the structure and nature of genetic diversity in germplasm collections is important for the efficient conservation and exploitation of plant genetic resources. The importance of using the genetic structure of germplasm collections in the selection of core collections has been stressed by several authors (Brown, 1989; Spagnoletti-Zeuli, 1993; van Hintum, 2000; Franco et al. 2006). Grouping accessions according to agro-ecological criteria is expected to enhance the possibility of recovering alleles responsible for local adaptability (Cordeiro et al. 1995). Knowledge of the structure of a germplasm collection can also be very useful for the optimization of its composition. Many collections of crop genetic resources have been established without a clearly defined conservation goal or mandate, which resulted in collections of considerable sizes, unbalanced compositions and high levels of duplication. Based on knowledge of the genetic structure, the representation of the different components of a crop's genepool can be adjusted to take care of over- or underrepresentation (van Treuren et al. 2009), thus ensuring that a genebank collection is not overburdened with large numbers of accessions that add little to the overall objective of conserving the maximum possible variation present in a gene pool. Based on groups formed by molecular markers, it is clear from this thesis (chapter 2 and 3) that there is an overlap in genetic diversity between coconut accessions from West Africa and those from Latin America and this information can be used for rationalization of the coconut collections in genebanks.

Genetic structure is also very important in association studies (Wang et al. 2005; Shriner et al. 2007). Correcting for population genetic structure or cryptic relatedness (unknown kinship among individuals) reduces rates of false positives in association studies (Pritchard et al. 2000b; Flint-Garcia et al. 2003; Zhu et al. 2008).

It should be noted, that genetic structures serve different purposes in core collection designation and in association studies. For designation of core collections, genetic structure guides the allocation of entries over the different groups (clusters), in which case it may be critical that accessions are clustered into discrete groups. Although the requirement for discrete grouping is often seen as convenient, in reality genetic diversity occurs in a continuum. However, many authors agree that stratification of germplasm collections leads to improvements of the quality of core collections (see Cordeiro et al. 1995; Franco et al. 2006). In association studies, fuzzy grouping can easily be accommodated since the main role of genetic structure is to indicate the relationships between individuals. In fuzzy grouping each accession is allowed to belong to more than one group, and associated with each accession is a set of membership probabilities (usually referred to as the Q-matrix) for the different groups. In association studies, the membership probability is used as an estimate of the contribution of each group to the to the genome of a given accession. Thus the Q-matrix allows modelling of the contributions of different groups to the genomes of individual accessions (Pritchard et al. 2000, Yu et al. 2006). In this thesis we proposed the use of co-phenetic distances between accessions as an alternative for incorporating relatedness information obtained from traditional clustering techniques in association studies (Chapter 2). The co-phenetic distance is the distance at which two accessions are clustered for the first time in a hierarchical cluster analysis. Co-phenetic distances may be considered as 'fitted' distances based on the dendrogram and as a consequence will contain less noise compared to observed, 'crude' distances. Relatedness based on co-phenetic distances can be used directly to correct for population structure or cryptic relatedness without a need for obtaining discrete groups. Studies need be carried out to establish the usefulness of co-phenetic distances in association studies. We need to answer questions such as: "is the co-phenetic distance a suitable estimate of kinship (usually referred to as the K-matrix) matrix in association studies?"

In the selection of core collections, a grouping is relevant only if it is meaningful in relation to evolutionary forces (e.g. natural selection, domestication, plant breeding etc.) that shaped the structure of genetic diversity. It is clear from the literature that when forming core collections most curators prefer to structure germplasm collections using passport data by means of a hierarchical branching method (see Brown, 1989a; van Hintum et al. 1995, 2000). This approach is simple and intuitive. In the hierarchical branching method, assumptions about and knowledge of the structure of a genepool is systematically used to split germplasm collections into smaller and smaller subgroups based on passport data until further splitting is not possible or is no longer relevant. Hierarchical branching is a form of a classification and regression tree - CART (see Berk 2008 for a detail description of CART). This approach was beautifully illustrated by van

Hintum et al. (2000) using the lettuce collection of the Center of Genetic resources, the Netherland (CGN) in which accessions were first grouped using domestication level (cultivated or wild), cultivated lettuces were further divided according to crop types (butterhead lettuce, cos lettuce, crisp lettuce etc.) and within each crop type accessions were divided based on area of origin (e.g. butterhead lettuce from Western Europe) and so on. However, often passport data are lacking or are of poor quality. We hope that the similarities between the groups formed by cluster analysis using molecular markers and groups based on passport data shown in chapters 2 and 3 provide assurance, and encourage researchers and curators to exploit the potential of molecular markers for understanding the genetic structure of germplasm collections. However, we do admit that there is still a big challenge in interpreting the meaning of the groups formed by molecular markers in their own right without making reference to other information such as passport data. At the moment it appears that the validity of groups formed by molecular markers depends on the ability of those groups to reflect the groups formed using other information sources (e.g. D'Hoop et al. 2010). We believe that as entire genomes get sequenced, the relevance of groups formed by molecular markers can be obtained without a need for comparison with other sources of information (we will come back to this point later). At this stage it would be interesting to determine how we can use different types of information (passport data, molecular markers etc.) simultaneously when studying genetic structure of germplasm collections. Through personal experiences and feedbacks from genetic resources users, most curators have accumulated a vast amount of knowledge which can be very valuable for understanding the genetic structure of germplasm collections and for the selection of core collections. How can such valuable information from curators be integrated with molecular and passport data? One way of integrating information from the different sources could be achieved by the use of a Bayesian approach. One could use passport data and information from curators as prior information when determining the genetic structure of germplasm collections using molecular marker data. Another possible alternative could be to use an approach similar to classification and regression trees (CART). In this case rather than using only passport data as a basis for hierarchically splitting germplasm collections in groups, one could also incorporate molecular marker information in the process. For example, in the case of the CGN lettuce collection, molecular marker information could be used to decide on whether the cultivated lettuce should first be split using crop types or using origin of accessions. By using molecular marker information it will be possible to determine which of the alternative splittings would lead to formation of groups which are genetically more distinct. Molecular marker information can also be used to determine when to stop further splitting in hierarchical branching process (i.e. molecular markers can be used for determining within group genetic diversity which can be used as a criteria for stopping further splitting).

The key question often asked after forming groups is what strategy should be adopted for deciding on the number of accessions to be selected from each group (i.e. allocation problem)? Different methods of allocation of entries over the different groups have been proposed and discussed in the literature (Brown 1989b). In general the importance of groups with respect to the purpose of core collections should determine the proportion of accessions to be selected from each group. For example if diversity associated with a given trait is suspected to be higher in a certain group then that particular group should be given more priority in allocation. In cases where allocation is based on genetic diversity within each group, there has to be a clear method for quantifying genetic diversity. Quantifying genetic diversity is much easier if the groups are formed using molecular markers than when using other types of data. When groups are based on passport data, genetic diversity is usually estimated using the history of domestication and dispersion of the crop (e.g. area of origins are thought to have more diversity than other areas). One could also use molecular marker information to quantify genetic diversity in groups formed using passport data.

6.3 Reference sets: Connections between germplasm collections in different genebanks using molecular marker data

Currently there are several international efforts (e.g. Generation Challenge Programme – GCP; http://www.generationcp.org) aimed at solving the problem of food insecurity using genetic resources available in genebanks around the world. The concept of reference sets of accessions and markers discussed in this thesis (chapter 4) can serve as a powerful method for connecting germplasm collections in different places and provide a global map of genetic diversity of a given crop leading to more efficient utilization of genetic resources. Through definition of overlaps between germplasm collections using molecular marker data, reference sets will allow these collections to be analyzed together, thus enlarging the space of inference. With just a few selected accessions and molecular markers, a reference set can provide an efficient way to relate new materials to existing collections and set up different crop-specific study panels that can be used by plant breeders worldwide. The method for selecting reference sets (GDOpt) discussed in this thesis can be applied in selecting subsets of accessions for creating the so-called MAGIC (Multiparent Advanced Generation Inter-Cross) population for QTL identification. Unlike the traditional mapping populations (e.g. RIL) which are obtained by crossing two lines only, MAGIC populations are established by crossing multiple founder lines. MAGIC populations are therefore more genetically diverse compared to the traditional mapping populations and are more suitable for QTL studies (see Kover et al. 2009). Since GDOpt selects a subset of accessions that maximally represent (based on the average distance between each accessions and the nearest entry) all the accessions in the collection, there is high probability that a subset selected using this method (GDOpt) captures multiple QTL alleles present in the germplasm collection. Another possible area of application of GDOpt could be in allele mining using the focused identification of germplasm strategy (FIGS (Mackay and Street 2004)). Based on information about the different groups of accessions in a germplasm collection, FIGS identifies a group or groups of accessions as candidates to be screened for alleles influencing a particular trait (Mackay and Street 2004). For example, to maximize the chances of finding functional diversity for powdery mildew resistance while limiting the number of wheat landrace accessions to a workable size, Bhullar et al. (2009) used FIGS to defined a subset of accessions for screening. The first step in identification of useful alleles can be done by screening a subset of accessions selected using GDOpt. The information obtained from screening a subset of accessions selected using GDOpt can then be used to identify other accessions (potential sources of desired alleles) from the whole collection to be screened (since each selected accession can be linked to non-selected accessions).

In attempting to setup a reference set (or core collection) using molecular markers a number of interesting questions will come up. Since different types of markers (SSRs, SNPs) often provide different types of information (see review by Vignal et al. 2002), how can we come up with robust reference sets? To what extent does a reference set or core collection formed using neutral diversity represent functional diversity? Functional genetic diversity is diversity that is directly associated with important traits. It is clear from the literature that in addition to crop evolutionary forces such as natural selection and domestication that affect neutral diversity, functional diversity is also shaped by plant responses to different environments as a form of local adaptation (see review on crop evolution by Burger et al. 2008). Can information accumulated from QTL mapping and allele mining studies be incorporated in the selection of reference sets (or core collection)? A number of allele-specific markers have been developed for marker-assisted selection in major crops such as rice and barley (see review by Kumar et al. 2010) and this information could potentially be used for the selection of reference sets/core collections or for the determination of the genetic structure of germplasm collections. In the light of our increasing knowledge of germplasm collections and diversifying interest of genetic resource users, we strongly believe that for a given crop, the concept of core collections or reference sets should be flexible so as to fulfill particular interests or roles in a changing environment. For example, as the genomes of different crops get sequenced, it should be possible to use GDOpt or other core collection algorithms to select subsets of accessions targeting specific sections of the genome say by giving more weights to molecular markers from those sections. However, such markers coming from specific sections of the genomes may sometimes show different levels of linkage equilibrium (correlation). At the moment most methodologies for analysis molecular marker data use the assumptions that markers are independent, and markers that are in linkage equilibrium are discarded. It is certainly interesting to quantity the amount of information that is lost by simply throwing away markers that show some evidence of correlation. For the case of functional markers, the loss of information caused by discarding correlated markers is likely to be higher than for neutral markers due to epistasis.

The concept of maximizing the representativeness of accessions in the whole collection which we emphasized in this thesis has largely been ignored by available methods for selecting core collections. We believe that core collections that maximize representativeness in relation to whole collection are often more robust and can serve multiple roles compared to core collections which are selected by maximizing specific genetic diversity parameters such as allelic richness or average distance between entries. For example, maximizing diversity could simply mean selecting accessions with extreme characteristics. Although these types of core collections (representing extremes) may be good for the specific roles for which they are created, a number of important questions should be answered before any attempt is made to use them for other purposes. Is it wise to put more or equal emphasis on outliers than common accessions? In general what would such a subset represent? With subsets of accessions that put much emphasis on extremes (which could be due to error), we risk representing a world that never existed in the first place. A breeder would certainly be interested in a subset of accessions with maximum number of functional alleles (preferably in an adapted background) but he/she is not interested in neutral alleles. For germplasm collections with a strong group structure, one question that is still open to debate is whether the selection of subsets of accessions should be based on richness of diversity within a group or on the degree of divergence between groups? Definitely further research is needed to determine the usefulness of subsets of accessions selected using different methods for mapping and breeding purposes. For example, if we are interested in selecting founder lines for establishing a MAGIC population which criteria (number of alleles, pair-wise distances between accessions, etc.) should a subset of accessions optimize? In addition, as more knowledge about the genomes of plants become available should the existing core collections be re-evaluated *e.g.* using functional markers, and their content modified?

6.4 Criteria for evaluation of core collections

In comparing the options for assembling core collections, one of the challenges is in deciding on the criteria for evaluating the quality of the resulting core collections. Of all aspects of core collection methodology, criteria for evaluating the quality of core collections has been given the least research attention. Apparently there are no clear guidelines for the choice of criteria for evaluating the quality of core collections and most researchers seem to choose criteria simply because those criteria were used in earlier publications. By relating evaluation criteria to the different types (objectives) of core collections, this thesis (chapter 5) hopes to help researchers to make appropriate decisions when selecting evaluation criteria. Since there is no one perfect core collection suitable for all purposes, it is important that one uses appropriate criteria if he/she is to get the best core collection for a given objective and avoid drawing false conclusions. In most cases once the criteria for the desired core subset is well defined, the selection of accessions can effectively and efficiently be handled as an optimization problem using algorithms such as MSTRAT (Gouesnard et al. 2001), PowerCore (Kim et al. 2007), Core Hunter (Thachuk et al.

2009) or GDOpt (Odong et al. 2011)). When a germplasm collection has been structured, the selection of accessions can be done by optimizing the desired criteria in the different groups and in that case the number of accessions to be selected from each group (allocation) will be determined by the relative importance of that group.

When evaluating the quality of core collections, most often the evaluation is done for each trait separately and later the results are combined with traits given equal weight (see Hu and Xu 2000; Tai and Miller, 2000). In this thesis we advocated the use of multivariate approaches (distance-based criteria). The distance-based criteria presented in this thesis are intuitive, easy to interpret and relate to the concept of representation of genetic diversity. When evaluating the quality of core collections we believe that not all traits or markers may deserve to be given equal weight. For example when evaluating core collections using phenotypic data, should traits with high heritability be given the same weight as traits with low heritability?

The most common criteria used in literature for evaluating core collections are based on summary statistics (means, variances, range etc.) (see Hu and Xu 2000; Tai and Miller, 2000). The main idea behind the use of criteria based on summary statistics is that the distribution of the traits in the core collection should reflect that of the whole collection. There is a conceptual problem with the statistical test used for comparing a core collection (sample) and a whole collection (population) in the literature. In a statistical comparison of the core and the whole collection, the question is not whether two samples are different, but could a sample (core) have been obtained from a particular population distribution (whole collection)? So we should be dealing with a one-sample test, and not a two-sample test.

6.5 Concluding remarks

The concept of core collections has finally come of age and the contributions of core collections to the utilization of plant genetic resources has been demonstrated. However, with increasing amounts of information being obtained from plant genome sequencing, new questions about the idea of core collections will certainly come up, and answers must be given. For example, will complete sequencing of genomes of crop species make the idea of core collections more or less relevant? This thesis has addressed among others the challenges faced when determining the structure of germplasm collections using molecular markers. We conclude from this thesis that a two-step approach (principal component analysis followed by Ward's cluster analysis) is suitable for unraveling the genetic structure of germplasm collections. We believe that the idea of reference sets of accessions and molecular markers will open a new avenue for sharing information between genebanks which will lead to a better utilization of genetic resources. The method of selection of accessions (GDOpt) proposed in this thesis will likely have extensive applications especially for the selection of lines for multi-parent crosses and allele mining. Finally we would like to stress that when selecting core collections or reference sets it is important that the objectives are clearly defined and such objectives should be the basis for evaluating the selected set.

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Summary

Genetic diversity of crop species stored in genebanks will play a vital role in addressing future, global challenges, especially those associated with the expected food crisis as a result of climate change and the fast growing world population. The effective and efficient management and exploitation of all available genetic resources depends largely on genebank managers and users (e.g. plant breeders) having a clear insight on the quantity and the structure of genetic diversity present in germplasm collections worldwide. The choice of methods for determining the genetic structure of germplasm collections using molecular markers is one of the challenges addressed in this thesis. In addition, the resources required for assembling, managing, conserving and providing access to the usually very large germplasm collections are limited. It is about three decades ago since the idea of core collections (*i.e.* a limited set of accessions representing the genetic diversity of a whole collection) was introduced to ensure efficient and effective management and utilization of the accumulated plant genetic resources with limited resources. In this thesis we expanded the idea of core collection from being a subset of accessions representing genetic diversity in a single genebank to a subset of accessions and molecular markers (reference set) for linking genetic diversity in different genebanks. The subset of accessions are selected from several genebanks. This thesis provides an extensive account of how to exploit the potential of molecular marker data for creating and evaluating core collections.

In chapter 2 we evaluated the appropriateness of traditional hierarchical clustering techniques (Ward and UPGMA) for determining the genetic structure of germplasm collections using molecular marker data. The performance of hierarchical clustering techniques was compared with that of STRUCTURE, a special model-based package designed for studying the genetic structure of natural populations. Based on our results, Ward performed much better than UPGMA in all aspects of determining genetic structure. In addition, groups formed by Ward were in agreement with groups formed by STRUCTURE and passport data. Using simulated data we showed that the co-phenetic correlation coefficient (one of the criteria for evaluating cluster analysis) is directly related to subgroup differentiation and consequently this criterion is a good indicator of the presence of genetically distinct subgroups in germplasm collections. However, our results also showed that for real data sets, the problem of determining the number of groups in the data set cannot be solved completely with traditional hierarchical clustering methods. The two-step approach we proposed and discussed in chapter 3 (see below) solved the problem of determination of number of groups in the data set.

Chapter 3 deals with a two-step approach for determining the genetic structure of germplasm collections. The first step involves applying principal component analysis to allele frequency data and using the Tracy-Widom distribution to determine the number of significant principal components. We then perform cluster analysis (Ward and model- based hierarchical clustering) using significant principal components only. This provided a tremendous boost in the performance of cluster analysis. No difference was found between Ward and model-based hierarchical cluster analysis. The two-step approach is very effective especially for determining the number of groups in the data.

In chapter 4 we studied statistical techniques for constructing representative subsets of accessions and accompanying sets of molecular markers that can be used to connect genetic resources from different genebanks. For the selection of accessions, we proposed Genetic Distance Optimization (GDOpt), a method which selects subsets of accessions that optimally represent all accessions. In terms of representing accessions not included in the subset, GDOpt performed better than existing core selection algorithms. However, by ensuring that the non-selected accessions are maximally represented by the selected accessions, the ability of GDOpt to obtain subsets which maximize genetic diversity parameters (for example allelic richness) is slightly compromised. For the selection of molecular markers we suggested the use of the backward elimination method (BE) or methods based on the first few principal component. In this thesis the ideal subset of molecular markers is defined as the one which maximally preserves the pair-wise relationships between accessions based on all molecular markers i.e. the pair-wise distances based on a selected subset of markers should have a high correlation with the pair-wise distances based on all the markers. In a fashion similar to the backward elimination method in multiple regression analysis, BE as defined for our purposes uses this correlation directly as the criterion for selecting markers. In the method based on principal components, molecular markers are selected based on their weighted sum of squared loadings on all principal components designated as important, in which the corresponding eigenvalues are used as weights. The current practice of using polymorphic information content (PIC) as a criterion for selecting molecular markers is insufficient when the interest is in a subset that preserves the main features of the genetic structure in the data.

Chapter 5 presents a critical examination of criteria for the evaluation of the quality of core collections. This chapter highlights the importance of selecting the right criteria when evaluating core collections. We defined different types of core collections and related each type with suitable evaluation criteria for quality. We proposed distance-based evaluation criteria and evaluated their performance using real data sets. The distance-based criteria not only allow the simultaneous evaluation of all variables describing the

accessions, but are also intuitive and interpretable, in contrast with the univariate approaches generally used for determining the quality of core collections.

Chapter 6 provides a general discussion that critically reflects on the concepts and methods used in this thesis and puts them into a broader perspective.

Samenvatting

De genetische diversiteit van gewassen zoals die wordt geconserveerd in genenbanken, is van cruciaal belang voor het aanpakken van problemen die in de nabije toekomst op wereldschaal zullen gaan spelen, vooral problemen die betrekking hebben op de verwachte voedselcrisis als gevolg van klimaatveranderingen en de sterke groei van de wereldbevolking. Het doelgericht en doelmatig beheer en gebruik van alle beschikbare genetische bronnen is in hoge mate afhankelijk van het inzicht dat curatoren en gebruikers van genenbanken hebben in de hoeveelheid en de structuur van de genetische diversiteit zoals die wereldwijd aanwezig is in genenbankcollecties. De keuze van methoden voor het in kaart brengen van de genetische structuur van collecties door het gebruik van moleculaire merkers, is één van de uitdagingen van dit proefschrift. Er zijn beperkte middelen beschikbaar voor het opzetten, beheren en conserveren, alsmede het toegankelijk maken van de veelal zeer grote gewascollecties. Om met beperkte middelen een doelgericht en doelmatig gebruik van al het verzamelde, genetische materiaal te garanderen is ongeveer dertig jaar geleden het idee van de 'core-collectie' geïntroduceerd. Een 'core-collectie' is een collectie van beperkte omvang die de genetische diversiteit van een gehele gewascollectie in een genenbank moet representeren. In dit proefschrift wordt het idee van 'core-collectie' uitgebreid van een representatieve deelverzameling van een gewascollectie in één genenbank naar een representatieve deelverzameling van gewascollecties van meerdere genenbanken. Hierbij worden moleculaire merkers gebruikt om de samenhang tussen de genetische diversiteit in de verschillende genenbanken te bepalen. In dit proefschrift wordt uitgebreid aandacht besteed aan het gebruik van het potentieel dat beschikbaar is in data van moleculaire merkers voor het opzetten en evalueren van 'core-collecties'.

In Hoofdstuk 2 wordt de geschiktheid onderzocht van traditionele, hiërarchische cluster technieken (Ward, UPGMA) voor het vastleggen van de genetische structuur van gewascollecties met behulp van moleculaire merkers. De resultaten van hiërarchische cluster technieken worden vergeleken met die van "STRUCTURE", een computer programma, gebaseerd op een statistisch model, dat speciaal is geschreven voor het bestuderen van de genetische structuur van natuurlijke populaties. Op basis van onze resultaten is duidelijk geworden dat Ward in alle aspecten van het vastleggen van genetische structuur veel beter presteerde dan UPGMA. Bovendien kwamen de clusters verkregen met Ward overeen met die verkregen met “STRUCTURE”, en met de paspoortgegevens van het geanalyseerde materiaal. Met behulp van computer simulatie laten we zien dat de co-phenetische correlatie coëfficiënt (één van de criteria voor het evalueren van cluster analyses) direct gerelateerd is aan de mate van differentiatie binnen gewascollecties, en daarmee een goede indicator van de aanwezigheid van genetisch te onderscheiden groepen in gewascollecties. Echter, onze resultaten laten ook zien dat voor praktijkdata, het probleem van het vaststellen van het aantal clusters niet volledig kan opgelost worden met traditionele hiërarchische cluster technieken. Als alternatief wordt hiervoor een twee-stappen benadering voorgesteld en besproken in Hoofdstuk 3.

In Hoofdstuk 3 wordt een twee-stappen benadering voor het vaststellen van de genetische structuur van gewascollecties gepresenteerd. De eerste stap betreft de toepassing van principale componenten analyse (PCA) op allel frequenties gevolgd door het vaststellen van het statistisch significante aantal principale componenten met behulp van de Tracy-Widom verdeling. De tweede stap betreft een cluster analyse (Ward en hiërarchische cluster analyse gebaseerd op een statistisch model), waarbij alleen gebruik wordt gemaakt van de statistisch significante principale componenten. Deze twee-stappen benadering leidt tot een enorme verbetering van de prestatie van cluster analyse, en laat geen verschil zien tussen Ward en hiërarchische cluster analyse gebaseerd op een statistisch model. De twee-stappen benadering is erg effectief, vooral in het vaststellen van het aantal groepen in gewascollecties op basis van data van moleculaire merkers.

In Hoofdstuk 4 worden statistische technieken onderzocht voor het construeren van kleine representatieve ‘core-collecties’ in combinatie met specifieke moleculaire merkers, die kunnen worden gebruikt om gewascollecties van verschillende genenbanken te verbinden. Voor het selecteren van accessies wordt “Genetic Distance Optimization” (GDOpt) geïntroduceerd, een methode die op zodanige wijze ‘core-collecties’ selecteert dat alle accessies optimaal gerepresenteerd worden. Vooral in de representatie van niet geselecteerde accessies presteert GDOpt veel beter dan bestaande methoden voor het selecteren van ‘core-collecties’. Echter, door het accent te leggen op de optimale representatie van niet-geselecteerde accessies, is GDOpt minder geschikt voor het selecteren van ‘core-collecties’, maximale genetische diversiteit (zoals “allelic richness”). Voor het selecteren van moleculaire merkers wordt achterwaartse selectie voorgesteld of methoden gebaseerd op de eerste paar principale componenten. In dit proefschrift wordt de ideale deelverzameling van moleculaire merkers gedefinieerd als die deelverzameling waarmee de paarsgewijze relaties tussen accessies gebaseerd op alle moleculaire merkers wordt behouden. In de praktijk betekent dit dat de paarsgewijze afstanden gebaseerd op een deelverzameling van merkers een hoge correlatie moet hebben met de paarsgewijze afstanden op basis van alle merkers. Net als bij de toepassing in multipele regressie, maakt achterwaartse eliminatie direct gebruik van de correlatiecoëfficiënt als criterium voor het selecteren van merkers. Bij de methoden gebaseerd op principale componenten worden de moleculaire merkers geselecteerd op basis van de gewogen som van gekwadrateerde ladingen van alle als belangrijk aangemerkte principale componenten, waarbij de corresponderende eigenwaarden worden gebruikt als gewichten. De huidige praktijk om de "polymorphic information content" (PIC) te gebruiken voor het selecteren van moleculaire merkers werkt onvoldoende als het gaat om het behouden van de belangrijkste aspecten van genetische structuur.

Hoofdstuk 5 bevat een kritische beschouwing van criteria voor het evalueren van de kwaliteit van 'core-collecties'. Dit hoofdstuk appelleert aan het belang van het gebruik van juiste criteria. Verschillende types 'core-collecties' worden gedefinieerd en aan elk type worden evaluatiecriteria gekoppeld. In dit hoofdstuk wordt voorgesteld om evaluatiecriteria te baseren op (genetische) afstanden tussen accessies; deze op afstanden gebaseerde criteria worden geëvalueerd op basis van hun prestaties met praktijkgegevens. De criteria gebaseerd op afstanden maken niet alleen simultaan gebruik van alle variabelen, zij berusten op intuïtie en zijn interpreteerbaar. Dit in tegenstelling tot benaderingen waarbij elke kenmerk apart wordt behandeld; deze worden in de huidige praktijk nog veel gebruikt voor het vaststellen van de kwaliteit van 'core-collecties'.

Hoofdstuk 6 bevat een algemene discussie waarin een kritische beschouwing wordt gegeven van de concepten en methoden die in dit proefschrift worden gebruikt, en waarin deze ook in breder perspectief worden geplaatst.

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List of Publications

Odong TL, van Heerwaarden J, Jansen J, van Hintum ThJL, and van Eeuwijk FA (2011a) Statistical techniques for defining reference sets of accessions and microsatellite markers. Crop Science

Odong TL, van Heerwaarden J, Jansen J, van Hintum ThJL, and van Eeuwijk FA (2011b) Determination of genetic structure of germplasm collections: are traditional hierarchical clustering methods appropriate for molecular marker data? Theoretical Applied Genetics 123(2):195-205: doi 10.1007/s00122-011-1576-x.

Odong TL, Jansen J, van Eeuwijk FA and van Hintum ThJL Quality of Core Collections for Effective Utilization of Genetic Resources *Review, Discussion and Interpretation*. Theoretical and Applied Genetics (*Under revision*)

Joost van Heerwaarden, **TL Odong**, FA van Eeuwijk Genetic differentiation and the use of PCA-based clustering for genotypic core selection. Theoretical and Applied Genetics (*Under revision*)

PE&RC PhD Education Certificate

With the educational activities listed below the PhD candidate has complied with the educational requirements set by the C.T. de Wit Graduate School for Production Ecology and Resource Conservation (PE&RC) which comprises of a minimum total of 32 ECTS (= 22 weeks of activities)

Review of literature (5.6ECTS)

- Structuring of genetic diversity of germplasm collections (2008)

Writing of project proposal (3.5 ECTS)

- Quantitative methods for sampling germplasm collections (2007/2008)

Post-graduate courses (3 ECTS)

- Genetic linkage mapping; Kyazma (2008)
- QTL Analysis: Kyazma (2008)
- Course: quantitative genetics with focus on selection theory ;the Graduate School, Wageningen Institute of Animal Science (2010)

Deficiency, refresh, brush-up courses (11.2ECTS)

- Modern statistics for life sciences (2008)
- Population and quantitative genetics (2008)

Competence strengthening / skills courses (3.9 ECTS)

- Endnote; personal study (2008)
- Interdisciplinary research: crucial knowledge and skills; WGS (2010)
- Mobilising your scientific network; WGS (2011)
- Project and time management; WGS (2011)

PE&RC Annual meetings, seminars and the PE&RC weekend (2.1 ECTS)

- PE&RC Weekend (2008, 2009)
- PE&RC Day (2008-2011)

Discussion groups / local seminars / other scientific meetings (11.2 ECTS)

- Statistical genetic colloquia (2008-2011)
- Biometris colloquium (2008-2011)

International symposia, workshops and conferences (10.7 ECTS)

- Generation challenge programme (GCP) workshop: reference sets of food crop germplasm for international collaboration; oral presentation; Montpellier (2008)
- Second IBS Channel network conference; oral presentation and one day pre-conference course (2009)
- Biometric section of Eucarpia; oral presentation and one day pre-conference course; Dundee, Scotland (2009)
- GCP Annual research meeting; oral presentation; Bamako, Mali (2009)
- *Arabis alpina* pereniality; annual research meeting; oral presentation; Grenoble, France (2012)

Curriculum Vitae

Thomas Lapaka Odong was born on 29th December, 1974 in Kalongo, Uganda. He attended Sacred Heart Seminary, Lacor (Gulu) and St. Peter's college (Tororo) for Ordinary (1990-1993) and Advanced (1994-1996) level education, respectively. Thomas graduated with BSc in Agriculture (1st class honours) from Makerere University, Uganda in 2000. Because of his love for Statistics he opted to do MSc in Applied Statistics (Biometry) from University of Kwa-Zulu Natal (by then University of Natal), South Africa which he completed in 2003. For his MSc thesis he worked on statistical methods for assessing variability in on-farm trials. In the academic year 2005/2006, he was a visiting Scholar at Michigan State University, USA where he took advanced level courses in theoretical and applied statistics. In December 2007, he started a PhD project which formed the basis of this thesis. Since 2005, he has attended several short courses mainly in statistical modelling, Linkage mapping, QTL analysis and analysis of next generation sequencing data. Thomas was involved in teaching Biometry and research methods at the school of agricultural sciences, Makerere University since 2003. At the same time he was also involved in providing technical backstopping in the area of study design and data analysis to both academic staff and postgraduate students. He also lectured at Uganda martyrs University, Nkosi and Gulu University. In October/November 2007 he was a visiting lecturer at the University of Zimbabwe where he taught applied statistics to postgraduate students in the department of Soil Science. Under the Regional University Forum for Capacity building in Agriculture (RUFORUM), Thomas was involved in training/re-tooling of academic staff in universities from East, Central and Southern Africa in research methods and applied statistics (2006/2007). He has participated in developing RUFORUM regional masters of science in Research Methods course currently being hosted by Jomo Kenyatta University of Agriculture and Technology (Kenya). He has also developed three training manuals in research methods and applied statistics. Since 2003, he has done consultancy work for several local and international research and development organisations including ASERECA, AT-Uganda, FAO-Sudan, International Potato Center (CIP Uganda office), PRAPACE (Uganda office) and RUFORUM amongst others. Since December 2011, Thomas has been a postdoc researcher in the Ecological and Evolutionary Functional Genomics Project, working on SNP discovery in *Arabis alpina* using next generation sequencing data.

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