

CHAPTER 8

CONCLUSIONS AND RECOMMENDATIONS

Scope and objectives of the study

Genetic variation is of fundamental importance in many areas of basic and applied biology. It is a prerequisite for breeding, collection and conservation strategies, as well as being the basis for genetic fingerprinting. The development of DNA-based marker systems such as RFLP, RAPD, AFLP and SSR, in particular, has made it possible to access the genetic variation in virtually any organism. Most DNA-based genetic diversity studies in maize to date, have focused on inbred lines rather than on out-bred populations. This is because the cost and complexity of the analysis process is lower in inbred lines than in populations. In order to analyze large numbers of out-bred varieties or populations, bulking or pooling strategies have to be employed (Kolliker *et al.*, 2001, Rebourg *et al.*, 2001). However, very limited information is available on the use of bulked DNA samples to estimate genetic variability and genetic relationships for out-bred maize accessions using molecular markers.

In the highland areas of Ethiopia, maize contributes greatly to household food security. Maize cultivars that are used in the highland regions are well adapted, but low yielding open-pollinated varieties resulted from centuries of selection. These accessions may be grouped into a smaller number of reproductively isolated populations, which may each have accumulated specific genetic adaptations for different highland conditions. Effective plant breeding and crop improvement programs depend on the availability of crop genetic diversity. In the search for diverse breeding materials, landraces are usually the major source of variation. This study represents a detailed investigation of maize accessions collected from the highlands of Ethiopia using morphological traits and

molecular markers.

Morphological variability

The morphological study revealed a wide range of variation among the highland accessions, which could be grouped into distinct phenotypic clusters. This allowed the selection of representative accessions from different highland areas of Ethiopia and from different morphological classes for molecular marker analysis. Specific recommendations from this phase of the study include that highland maize breeders in Ethiopia should give importance to kernels per row as a selection criterion to increase grain yield, because simple phenotypic selection of plants bearing higher numbers of kernels per row may lead to trait improvement of up to 37.8%.

Use of bulked leaf samples for genetic diversity studies

Individual and bulked - AFLP analyses suggested that bulking equal amounts of leaf samples from 15 individual plants per accession was an effective means of producing representative profiles of the out-bred varieties, thereby reducing the cost of DNA extraction and subsequent marker analysis. Cluster analysis of AFLP data grouped most of the Northern accessions into one cluster, while the Western and Southern accessions were grouped together. Partitioning of the total genetic variability into within and between agroecologies revealed substantially higher variability within than between agroecologies. The consistency of the results between Wards clustering method and partitioning of variation showed that bulked-AFLP marker analysis is

useful for studying the genetic diversity and relationships of out-bred crop varieties or populations.

In another experiment, we validated the genetic relationships detected by AFLP analysis with bulked SSR analysis. Analysis of individuals included in the bulk showed that an allele is generally detectable in the bulked sample when present in a proportion greater than 1 out of 15 (7%) of the individuals within DNA pooled-samples. Similar to the result of the AFLP analysis, cluster analysis performed on SSR data showed that accessions collected from the Northern agroecology were clearly distinct from the Western and Southern accessions, but there was very little differentiation between the Western and Southern accessions. These results indicated that the two techniques revealed similar genetic patterns in traditional Ethiopian highland maize accessions and are useful to study genetic diversity in out-bred varieties or populations. However, bulked SSR analysis is more useful than bulked – AFLP because SSR map positions are frequently known, which is useful to associate traits of interest with molecular markers and to compare association study result across different studies.

Genetic diversity in traditional Ethiopian highland maize accessions

High mean genetic diversity values were obtained in the traditional Ethiopian maize accessions with both marker systems. The values of overall genetic diversity obtained in this study agreed with diversity values reported in other studies of out-bred maize populations collected from Europe using RFLP (Rebourg *et al.*, 2001 mean, 0.55) and

from tropical and sub tropical maize populations and synthetics using SSR data (Pinto *et al.*, 2003 mean, 0.48). Therefore, it is possible to conclude that Ethiopian traditional maize accessions may be as diverse as European and tropical maize populations and synthetics. The presence of such variation suggests (1) adaptation of these accessions to many niches of Ethiopian highlands over a long period of time and (2) that highland farmers have maintained a wide diversity of maize accessions to meet their social, economic, cultural and ecological needs. In fact, farmers' selection for desirable agronomic traits may be the main force in shaping the genetic dynamics of the highland maize accessions. Landraces and farmers are interdependent and both are in need of each other for their survival (Appa Rao *et al.*, 1998).

In all studies (morphological, AFLP and SSR), differences in genetic diversity measures were detected among agroecologies (Table 6.4). These results suggest that farmers' selection criteria are different for the different agroecologies in accordance with the specific environmental conditions and/or local environmental condition may have influenced the genotypic constitution of these accessions. Farmers in the Northern agroecology have selected plants with short stature, short flowering time and that mature earlier to synchronize with moisture stress and short growing periods which are prevalent in the Northern agroecology. However, farmers in the Western and Southern agroecologies have selected tall, broad leaf and late maturing maize accessions, because these regions have higher rainfall, more fertile soils and longer growing periods (EARO, 2000). This underscores the importance of considering indigenous knowledge of genetic diversity in attempts to collect and evaluate local accessions.

Implication for breeding and conservation

A combination of morphology and molecular analyses revealed three groups of maize accessions, which could be useful to design different breeding programs for the highlands of Ethiopia. The first group (Figure 6.3, I) constitutes the early maturing, short statured accessions which were collected from the Northern agroecology from which they probably acquired earliness. This group is of special interest to maize breeding in Ethiopia where drought is one of the key production constraints. Therefore, these materials could be used as basis for the development of drought tolerant cultivars and need to be given a special emphasis in further crossing programs. The second group (Figure 6.3, II) includes the tall, high yielding varieties which are currently the most important landraces grown in the Southern and Western parts of Ethiopia. Therefore, accessions in this group can be used for the development of high yielding varieties suitable for high potential maize growing regions of Ethiopia. The third group (Figure 6.3, III) includes tall, late maturing and low yielding accessions, which are being cultivated in some parts of the Northern, Western and Southern highlands of Ethiopia and might be used as source of fuel (dry stalk).

The highland collection of maize accessions is maintained at the Institute of Biodiversity Conservation (IBC), Ethiopia. These 287 landraces were collected from all over the country. These accessions contain high genetic diversity as can be seen from morphological and molecular diversity measured in this study. For example, the Northern accessions are able to grow and produce under very harsh environmental conditions (drought, poor soils, excessive radiation, etc) and have relatively good

yield. On the other hand, the Western and Southern maize accessions have evolved with the parasitic weed *Striga spp* for several hundred years and are able to produce higher yield. In Africa, this weed is a significant pest of maize and sorghum, and to date, little resistance has been reported in maize (Hoisington *et al.*, 1999). Thus, Ethiopian highland maize accessions could be a source of variability for weed tolerance (*Striga spp.*), drought and cold tolerance. More accessions should be collected from all agroecologies to capture these valuable and unique germplasm.

Association mapping and the effect of admixture

The results of a preliminary association study using SSR marker alleles revealed putative associations of SSR markers with quantitative variation among traditional Ethiopian highland maize accessions. These results will assist the selection of divergent parental accessions for the development of inbred lines, thereby maximizing heterosis in future hybrid-breeding programs. Significant differences in alleles frequency were observed between the agroecologies (except between the Western and Southern) and most likely resulted in some cases from the selection for traits that contribute to overall performance and adaptation. The study also gives a preliminary method for selection of useful accessions prior to field evaluation, which is costly and time consuming. For example, the SSR allele *phi037-3* was associated negatively with flowering time, tallness and maturity. This allele also had positive and significant effects for grain yield and 1000 seed weight and number of kernels/row. Therefore, this allele can be used to develop short maturing plants without significantly reducing grain yield.

However, one of the problems of association genetic studies is the presence of population substructure (admixture) as observed in this study, which causes the detection of false positives. In this study, two groups of population structure were observed. The first group was accessions collected from the Northern agroecology and the second group was accessions collected from the Southern and Western agroecologies. Out of 98 SSR alleles scored in this study, eight individual alleles were fixed in the Northern accessions and 12 alleles were fixed in the Western and Southern accessions (Chapter 5). Hence, these 20 alleles could be significantly associated (by chance) with any traits that are different between the two groups. Therefore, future population level association studies should consider the population structure in order to increase the power of detecting true markers associated with traits of economic importance.

In conclusion, this research is the first attempt to characterize the genetic diversity found within traditional Ethiopian highland maize accessions. Maize breeders in Ethiopia will benefit from knowledge of the genetic relationships of the highland maize accessions so that they can improve adaptive and agronomic traits through traditional hybridization and/or marker-assisted selection techniques. The analytical tools outlined in this dissertation can be useful for detecting genetic variation among open pollinated crop varieties and will aid in the conservation and preservation of unique genetic diversity present in germplasm collections. Production stability and global food security are linked to the conservation and exploitation of worldwide genetic resources and this research attempts to add to that body of knowledge.