# 7. Summary

The ‘breeder’s exemption’ as fixed in the UPOV convention on plant variety protection allows the use of protected germplasm for the development of new plant varieties. The aim of this concept is the creation of new genetic variation to guarantee a continuous breeding progress. However, the use of molecular markers in backcrossing programs and genetic engineering has created the technical basis to develop new plant varieties without original breeding efforts. Therefore, the concept of ‘essential derivation’ was implemented into the 1991 Act of the UPOV convention to distinguish between varieties that resulted from intensive and creative selection programs and cultivars that were developed without major genetic changes from these former varieties. Accordingly, a variety is deemed to be essentially derived from an initial variety (IV), if it (i) was predominantly derived from the IV, (ii) is clearly distinguishable from the IV, and (iii) genetically conforms to the IV in the expression of it’s essential characteristics.

The goal of this thesis was to evaluate and compare different approaches to assess conformity in the expression of the essential characteristics between IV and essentially derived varieties (EDVs) and to derive a theoretical and experimental basis for the development of thresholds to distinguish between independently derived varieties and EDVs in maize (*Zea mays* L.). The main focus was set on the evaluation of genetic distances based on ‘simple sequence repeats’ (SSRs) and ‘amplified fragment length polymorphisms’ (AFLPs) as well as the factors contributing to the GD between parental inbreds and their progeny lines. Furthermore, the ability of heterosis and morphological distances for identification of EDVs was examined. In detail, the objectives were to (1) investigate genetic and technical sources of variation in data derived from simple sequence repeats (SSR) and amplified fragment length polymorphism (AFLPs) within maize inbreds and assess their impact on identification of EDVs, (2) analyze the factors influencing genetic distances (GD) based on SSRs and AFLPs between related maize inbred lines, (3) investigate the power of SSR- and AFLP-based GD estimates, morphological distances and heterosis for discriminating between progenies derived from $F_2$, $BC_1$, and $BC_2$ populations, (4) exemplify theoretical and simulated results with experimental data, and (5) draw conclusions with regard to EDV thresholds suggested in the literature.

A total of 220 flint, dent, and US maize inbred lines was genotyped with 100 SSRs equally distributed across the maize genome. The 220 lines comprised 163 triplets. A triplet consisted of one progeny and both parental lines, where the former was developed from an $F_2$-, $BC_1$-, or $BC_2$ population. A subset of 58 lines (38 triplets) was genotyped additionally with 20 AFLP primer combinations. Furthermore, morphological traits and heterosis were observed for these 38 triplets in a field experiment over two years and three locations.

In addition, two to five accessions from nine inbred lines and five doubled haploid (DH) lines were taken from different sources or drawn as independent samples from the same seed lot and genotyped with SSRs and AFLPs to examine the variation of SSR and AFLP data within maize inbred lines. The GD between accessions of the same inbred or DH line amounted to 0.03 for SSRs and to 0.01 for AFLPs and was, therefore, of minor importance for identification of EDVs.

Parental genome contribution to $F_2$-derived lines estimated with SSRs ranged from 0.25 to 0.70 with a mean of 0.49. Deviations of the mean from the expected value can be explained by the occurrence of non-parental bands that were detected in 4% of all datapoints. The parental contribution of the recurrent parent to $BC_1$-derived progenies varied from 0.44 to 0.79 with an average of 0.64 and was significantly smaller than the expected parental genome contribution of 0.75. The distributions of GD values for parental lines and their $F_2$- and $BC_1$-derived progeny overlapped for simulated as well as for experimental data.

An analysis of variance revealed that for $F_2$-derived progeny lines 34% of the variance of the GD between parent and progeny line ($\sigma^2_{GD_{(P1;O)}}$) were explained by the variance of the GD between the parental lines ($\sigma^2_{GD_{(P1;P2)}}$) and 66% by the variance of the parental contribution ($\sigma^2_p$). For $BC_1$-derived progenies, $\sigma^2_{GD_{(P1;O)}}$ was largely independent of $\sigma^2_{GD_{(P1;P2)}}$, as more than 95% of $\sigma^2_{GD_{(P1;O)}}$ were explained by $\sigma^2_p$.

Assuming that the derivation of a line from an $F_2$ population was an accepted breeding procedure and the derivation from a $BC_1$ population would not be accepted, we observed Type II errors (β) ranging from 0.23 to 0.37 depending on the germplasm pool for a given Type I error (α) of 0.05. For a threshold between $BC_1$ and $BC_2$, β ranged from 0.40 to 0.60 with an increasing tendency for higher BC levels. For fixed GD thresholds of

T=0.25, 0.20, 0.15, and 0.10 suggested in the literature, substantial differences for α and β were found between different germplasm pools. Therefore, thresholds need to be gene pool specific and different thresholds for potential EDVs from intra-pool crosses than for progenies from inter-pool crosses must be applied.

Discrimination of $F_2$-, $BC_1$-, and $BC_2$-derived progeny lines on the basis of heterosis and morphological distances revealed β values ranging from 0.50 to 0.95 depending on the trait or combination of traits. Therefore, heterosis and morphological distances were fairly inappropriate tools for identification of EDVs due to the larger overlaps of $F_2$-, $BC_1$-, and $BC_2$-distributions compared to GDs based on molecular markers.

In general, SSRs and AFLPs were the most adequate tools to uncover close pedigree relationships between maize inbred lines and to discriminate among lines derived with accepted or non-accepted breeding procedures. Therefore, the results presented in this study provide an example for identification of EDVs and can be transferred to other diploid crops by adjusting the corresponding thresholds.