6. General discussion

The Concept of Essential Derivation within the Tendencies in Modern Plant Breeding

A selection for simple and complex traits to improve domesticated animals or plants in highly developed long-term selection processes has for centuries been performed entirely on their phenotypes. Even though this has proven to be a fabulously successful approach, the forthcoming age of biotechnology and genomics offers the prospect of shifting selection gradually from phenotypes to genotypes (Walsh, 2001). In addition, the available genome sequence of *Arabidopsis thaliana* (The Arabidopsis Genome Initiative, 2000) as well as the growing number of identified genes in major crops, such as *brachytic* (Multani et al., 2003) or *dwarf8* (Thornsberry et al., 2001) in maize (*Zea mays* L.), have provided plant breeders with new perspectives as the exploitation of sequence homologies with other crops or inter-specific introgression of favorable genes are (Walsh, 2000).

In combination with already available tools like marker-assisted selection, these new approaches steadily reduce the time intervals necessary for breeding new varieties. This increases the pressure on plant breeders to release new varieties to keep up with the breeding progress of competitors. Therefore, marker and sequence information, cloned genes, germplasm resources as well as protected germplasm must be available to all breeders, ensuring the most efficient breeding progress to the breeders of all crops. The concept of essential derivation, as implemented in the 1991 Act of the UPOV convention (UPOV, 1991), is thus a first step towards a framework for regulations of the exchange of germplasm among breeders and could be followed by regulations for exchange of marker information or DNA-sequences.

Identification of EDVs and Rating of Threshold Scenarios

Since its implementation in 1991, the EDV concept has gained explicit proponents as well as severe criticism. Troyer and Rocheford (2002) pled for low EDV thresholds ($T \leq 0.10$) with only low royalty fees to be paid by the breeders of EDVs over a short period of time because higher thresholds would cause more EDVs and fewer IDVs. As a result, more EDVs would give rise to more lawsuits and more royalty payments and thus more

money for lawyers and accountants. Investments in the germplasm development would consequently be limited and the rate of yield improvement would be expected to decrease (Duvick, 1984). Nevertheless, the number of intellectual property lawyers in the USA is growing faster than the amount of research (Barton, 2000).

In contrast, the International Seed Federation (2002) strongly supports the EDV concept favoring thresholds from approx. GD=0.20 to 0.25, as suggested by Smith and Smith (1989), because it allows taking the above mentioned new technological developments into account. Furthermore, a strict threshold would support classical "creative" plant breeding and prevent from plagiarized "cosmetic" breeding, without hindering additive improving plant breeding. It would also enable building up a legal basis for balanced agreements among breeders as well as between breeders and inventors of patented procedures or products.

We have shown that Type I (α) and Type II (β) errors of a given GD threshold T were dependent on the crop, the degree of polymorphism of the marker system within the particular germplasm pool, the set of markers used, and the applied distance measure (Heckenberger et al., 2004). For example, T=0.25 based on Rogers' distance resulted in a fairly low α=0.07 to detect a BC_1-derived dent line as an EDV and would, therefore, be a possible EDV threshold to discriminate F_2- and BC_1-derived dent lines. For flint lines, however, T=0.25 yielded a considerably higher α=0.18. A possible threshold to distinguish between BC_1-, and BC_2-derived flint lines would be T=0.10. For dent and introgression lines, α-values were smaller than 0.02 and β_T lower than 20%. This indicates that a threshold of T=0.10 would be too conservative to distinguish between BC_1- and BC_2-derived dent or introgression lines and would consequently state the development of a BC_2 to a protected variety as an accepted breeding procedure (Troyer and Rocheford, 2002).

In detail, ASSINSEL and SEPROMA proposed a two-stage threshold for the detection of EDVs with a "red zone" of $GD_{(P1,O)}<0.10$, where a variety should be judged as an EDV, a "green zone" of $GD_{(P1,O)}>0.15$ (SEPROMA) or $GD_{(P1,O)}>0.20$ (ASSINSEL), where a variety should be judged as an IDV, and an "orange zone" between the two particular thresholds, where additional information is necessary to decide whether a variety is essentially derived or not. For the proposal of ASSINSEL, this would indicate that breeding an F_2-derived progeny from a protected line would be an accepted breeding procedure, but 72%, 39%, and 19% of flint, dent, and introgression BC_1-progenies would fall into the

"orange zone". The proposal of SEPROMA would indicate that breeding a BC_1-derived progeny from a protected line would be accepted with 36%, 13%, and 7% of flint, dent, and introgression BC_2-progenies situated in the "orange zone". For the "red zone" of ASSINSEL and SEPROMA, the same conclusions hold true as stated above for T=0.10.

Threshold Scenarios already applied on other Crops

In contrast to the presented scenarios based on the construction of frequency distributions of GD for each particular level of relatedness, the breeders of lettuce (*Lactuca sativa* L.) adopted a different scenario for essential derivation (International Seed Federation, 2003) based on a reference set that represent the total of all protected lettuce varieties. They agreed that a variety is deemed to beessentially derived if its GD to the initial variety was smaller than 95% of all the pairwise GDs of the reference set, independently of the marker system or the marker set used. For a recommended standard set of AFLP primer combinations, this threshold amounts currently to GD=0.05 based on 1- Jaccard's (Jaccard, 1908) similarity coefficient.

In ryegrass (*Lolium perenne* L.), a provisional threshold of a squared Euclidean Distance of seven was adopted in 1999 with the intention of a critical review after five years. If the distance was seven or lower, the breeder of the IV may ask for ISF arbitration. The breeder of the putative EDV will have to show that he has not practiced essential derivation from the IV. The arbitrators also have the right to ensure that the putative IV is not itself an EDV from a preexisting variety (Roldan Ruiz et al., 2000a).

All above mentioned threshold scenarios, including the scenarios developed in this study, depend more or less on the choice of reference sets of varieties or inbred lines to adjust the thresholds according to a crop or germplasm pool. The choice of varieties to be included into reference sets for the development of thresholds is, therefore, a crucial issue for the identification of EDVs. As genetic diversity within a certain crop may differ between countries or growing regions, a creation of the reference sets, representative for the crop or germplasm pool, may lead to problems. Consequently, thresholds should also be specific for the region they are developed for and the set of reference varieties must be assembled with caution.

Influence of intra-varietal Variation and Lab Error on the EDV Concept

A considerable variation between accessions of the same maize line, caused by lab error, PCR artefacts, or heterogeneity within varieties due to mutation or outcrossing, was observed for both SSRs and AFLPs (Heckenberger et al., 2002; 2003). Our results for SSRs confirmed a study of Gethi et al. (2002), who reported a variation in SSRs of approx. 8% between sources of the same inbred line. In addition, Vigouroux et al. (2002) and Matsuoka et al. (2002) reported considerable mutation rates, particularly for SSRs with a di-repeat motif, that were higher than expected for the natural mutation rate of genomic DNA.

Within parent-progeny triplets, this intra-varietal variation leads primarily to non-parental alleles (NPAs) in progeny lines. For SSRs, NPAs were found for 4.2% of all progeny data points, which was considerably lower than reported by Bernardo and Kahler (2001). The size differences from the corresponding parental alleles ranged from 1 to 81bp with a mean of 14bp, but a considerable portion of NPAs differed only 1-3bp from their corresponding parental alleles and could, therefore, be re-scored and assigned to their parental alleles. NPAs were detected in 2.2% of all AFLP progeny data points, whereas 45% of all AFLP markers showed an NPA in at least one triplet. In addition, the number of NPAs per triplet was highly correlated between SSRs and AFLPs.

The occurrence of NPAs decreases the correlation between the marker-estimated GD and the true GD, and should, therefore,be avoided as much as possible. Non-parental alleles were observed for a higher percentage of SSRs than of AFLPs, due to the lower error rate of a dominant marker system such as AFLPs (Heckenberger et al., 2003). In addition, the frequency distribution of size differences between NPAs and their corresponding parental alleles indicates that NPAs for SSRs were mainly caused by artificial stutter bands (Smith et al., 1997) or 1bp-differences between a parental and a progeny allele (Heckenberger et al., 2002). Hence, we recommend to avoid the use of SSRs with di-nucleotide repeat motifs for identification of EDVs, to minimize the probability of the occurrence of stutter bands and to reduce the risk of mutations (Vigouroux et al., 2002). However, the influence of NPAs on the selectivity of a particular marker system was rather small, and could be neglected after a cautious re-scoring of data.

For closely related genotypes, intra-varietal variation generally leads to an over-estimation of GDs, to the benefit of the breeder of the putative EDV. The breeders of the IVs should, therefore, warrant a high level of homogeneity in their inbred lines for their own benefits. Consequently, we strongly recommend increased levels of homogeneity of maize inbred lines before applying for plant varietal protection, as well as replications of lab assays to minimize experimental errors.

The use of Molecular Markers for DUS Testing

In several crops, *e.g.*, oilseed rape (*Brassica napus* L.) (Lombard et al., 2000), soybean (*Glycine max* L.) (Giancola et al., 2002), and maize (Dillmann et al., 1997), the use of molecular markers for testing of distinctness was evaluated. A common result of all the above-mentioned studies, including the present thesis, was that cultivars indistinguishable by morphological descriptors differed considerably in their banding patterns revealed by molecular markers.

This indicates that molecular markers offer the possibility for a more accurate comparison of varieties than morphological traits do. These comparisons, however, might be too accurate to observe distinctness on the basis of single bands because of the limited reproducibility of molecular marker data due to PCR artifacts. Consequently, observing distinctness on the basis of molecular markers would require certain thresholds for distinctness, similar to EDV thresholds to observe on conformity. All the above-mentioned authors suggested, therefore, the use of phenotypic characters for DUS testing with only an additional application of molecular markers.

Factors influencing the Relationship between *f* and GD

The coancestry coefficient (*f*) (Malécot, 1948) between parental lines and progenies was used in this study as a benchmark for breeding procedures applied for breeding the in the derivation of progenies. Factors influencing the relationship between *f* and GD were considered important for the validation of the ability of GDs to identify EDVs.

The value of *f* is defined as the probability that two homologous genes taken at random, one from each individual, are identical by descent (*ibd*), *i.e.* they are copies of the same gene from a common ancestor. In contrast, the Genetic Similarity (1-GD) of lines is based on bands alike in state (*ais*), *i.e.* bands indistinguishable whether they are identical due to a common ancestor or due to the genetic background of the particular germplasm. Bands that were only *ais* but not *ibd*, subsequently designated as *oais*, were ignored in calculating *f*, but remain considered for the calculation of GDs as they were indistinguishable from genes that were *ibd*. Consequently, for a close relationship between GD and *f*, the fraction of bands *oais* should be small (Messmer et al., 1993).

As only GDs based on bands *ais* can currently be calculated, we used $\hat{\mu}_{GD_{(P1,P2)}}$ of unrelated lines (*f*=0) within the same material group or germplasm pool as an estimator of the proportion of bands being *oais*. A method that might unravel the proportion of bands *ibd* and the conditional proportion of bands *oais* was proposed by Bernardo et al. (1996) using an iterative approach on the basis of known parent-progeny relationships. Nevertheless, the estimation of identity by descent with poorly or unknown pedigree relationships, as in the case of EDV, remains an unsolved problem.

In addition, *f* is based on several simplifying but mostly unrealistic assumptions (Melchinger et al., 1991). The first assumption (all lines in the pedigree pathway are homogeneous and homozygous) may be justified for most of the highly inbred lines in this study, but may not be true for all lines used. For some lines examined in this study, up to 25% heterozygous SSR loci were detected, although they were highly inbred. Violation of the second assumption (lines with no common parentage have *f*=0) leads to an underestimation of *f* if progenitors are, in fact, related. The third assumption (lines derived from a cross obtained half of the genome from each parent) is most disputable, as observed in the present study.

Furthermore, the relationship between *f* and GD is affected by selection, drift, and mutation. As the genetic model used for the simulation study was allowing for drift, but not for either selection or mutation, the good fit of observed and simulated data for F_2-derived progenies indicates, that the variation in *p* for F_2-derived lines was mainly caused by genetic drift, whereas the influences of selection or mutation on *p* were negligible. This result was in agreement with the results published by Bernardo and Kahler (2001), who reported

that the mean parental contribution for unselected F_2-progenies was close to the expected value of 0.50. Moreover, they found that the selection of progeny lines tended to increase the frequency of alleles of the parent selected for, whereas no significant differences with unselected progenies were observed.

The Use of Computer Simulations

The present triplet studies were carried out with empirical distributions for GD values between parents and progenies ($GD_{(P1,O)}$). Due to a limited number of F_2-, BC_1-, and BC_2-derived progenies, an analytical description of the distribution of $GD_{(P1,O)}$ is not yet available. Simulations, as evaluated by Bohn et al. (2004) in a companion study, were, therefore, used as an alternative approach to derive the distribution of the test statistic. The simulations were conducted using the PLABSIM software package (Frisch et al., 2000). This software enables to flexibly alter different crop or genome specific parameters, such as chromosome length, marker density, or degree of polymorphism of applied markers. Their effect on the distribution of $GD_{(P1,O)}$ can, thus, be directly assessed.

Simulated $GD_{(P1,O)}$ values were calculated on the basis of mean and variance of GD values between unrelated lines of a particular germplasm pool. Depending on the accuracy of the estimation of $\mu_{GD_{(P1,P2)}}$ and $\sigma^2_{GD_{(P1,P2)}}$, the simulations proved to be a powerful tool to verify empirical distributions of $GD_{(P1,O)}$. Moreover, the simulations can be applied to simulate EDV scenarios for any diploid crop, if parameters $\mu_{GD_{(P1,P2)}}$ and $\sigma^2_{GD_{(P1,P2)}}$, as well as the number of chromosomes and the chromosome length are known accurately, even when no empirical data of $GD_{(P1,O)}$ is available.

Direct *vs.* indirect Measures of Conformity

Additionally to the use of molecular markers, which was proposed for identification of EDVs by various authors (Bernardo and Kahler, 2001; Dillmann et al., 1997; Roldan Ruiz et al., 2000a; Smith and Smith, 1989), the use of phenotypic descriptors, such as morphological traits or heterosis, is still under consideration (ASSINSEL, 2000; Roldan Ruiz et al., 2000b; Gilliland et al., 2000; International Seed Federation, 2002; Smith and Smith,

1989). Supporters of phenotypic data application claim that the term 'conform in the expression of its essential characteristics that result from the genotype' (UPOV, 1991) implies the use of phenotypic data rather than molecular data.

Numerous studies showed a triangular relationship between GDs based on molecular markers and morphological distances (MDs) based on phenotypic traits (Burstin and Charcosset, 1997; Dillmann and Guérin, 1998; Rebourg et al., 2001; Roldan Ruiz et al., 2000b). By contrast, several studies reported linear relationships and high correlations between GDs and mid-parent heterosis (Melchinger, 1999; Boppenmaier et al., 1993; Smith and Smith, 1989; Ajmone Marsan et al., 1998).

Opponents of the use of phenotypic data state that even highly heritable phenotypic traits can offer only a rough estimate of the true relatedness of two cultivars. Based on our results, GDs based on molecular markers have clear advantages for the identification of EDVs. First, molecular data provide a direct estimate of the true relatedness of two genotypes because they are unbiased by the environmental effects. Second, molecular data reflect the percentage of the genome in common between the IV and a putative EDV, whereas certain morphological traits may differ in their expression within different environments, thus requiring extensive field trials. Third, a large number of markers is available for genotyping cultivars of all crops, but only a limited number of morphological traits can be observed with reasonable financial and labor efforts. Forth, only a small part of the genome might be involved in the expression of morphological traits, whereas markers can be chosen explicitly to ensure an equal and dense coverage of the genome. Fifth, scoring of marker bands can be automated to a large extent (Ziegle et al., 1992), thus being objective and reproducible, whereas morphological data may vary due to the subjectivity of the scoring person(s) (Nuel et al., 2001). Having all those issues in mind, we suggest a redefinition of the term "essential characteristics" in the sense that marker bands can also be regarded as essential characteristics in the terms of the UPOV convention (UPOV, 1991).

Accepted *vs.* non-accepted Breeding Procedures

No agreement on accepted or non-accepted breeding procedures has been achieved so far in maize. Studies on the influence of somaclonal variation during transformation yielded high similarities between transformed maize lines and their isogenic non-transformed counterparts (Marhic et al., 1998; Murigneux et al., 1993). This indicates that transformed varieties will most likely be judged as EDVs (Borgo et al., 2002) from their isogenic counterparts, even if they are distinct from them.

Regarding the number of acceptable backcrosses, proponents of low GD thresholds (*e.g.,* GD≤0.15) state that the original UPOV convention gives the term "backrosses" in its plural form in the examples of breeding procedures yielding EDVs (ASSINSEL, 1999), indicating that at least one backcross to a protected variety should be accepted. In contrast, opponents of low GD thresholds argue that by developing a BC_1, up to 95% of the genome of the recurrent parent can be maintained by marker assisted selection, which is against the intention of using a variety as a source of initial variation. However, no consensus has currently been achieved.

Conclusion and Outlook

The present thesis provides the first detailed comparison of various distance measures on their ability to identify EDVs in maize. We have shown that for various reasons GDs based on molecular markers are superior to MDs or heterosis in reflecting the true genetic relationships of two cultivars. Consequently, procedures for the identification of EDVs should be developed with an emphasis on molecular marker technologies, rather than on phenotypic traits.

For future prospects, a growing number of markers will be available for each marker system, ensuring an increased precision of GD estimates (Foulley and Hill, 1999) and, therefore, reducing the probabilities of being judged for essential derivation by chance. In addition, new marker systems and techniques, such as single nucleotide polymorphisms (SNPs), or expression profiles in combination with microarrays or DNA chips will further increase the accuracy of molecular methods in estimating the true genetic relatedness of two cultivars. Finally, an adapted form of forensic approaches (Gill et al.,

1995; Graham et al., 2000), already applied successfully in human genetics for verification of parentage or disproving suspects, could aid in the identification of EDVs.

References

Ajmone Marsan, P., P. Castiglioni, F. Fusari, M. Kuiper, and M. Motto. 1998. Genetic diversity and its relationship to hybrid performance in maize as revealed by RFLP and AFLP markers. Theor. Appl. Genet. 96:219-227.

ASSINSEL. 1999. Essential derivation and dependence. Practical Information.

ASSINSEL. 2000. DUS testing: phenotype *vs.* genotype. ASSINSEL, Position paper adopted at the Rome Congress in May 2000.

Barton, J. H. 2000. Intellectual property rights: reforming the patent system. Science. 287:1933-1934.

Bernardo, R. and A. L. Kahler. 2001. North American study on essential derivation in maize: inbreds developed without and with selection from F_2 populations. Theor. Appl. Genet. 102:986-992.

Bernardo R, Murigneux A, and Karaman Z. 1996. Marker-based estimates of identity by descent and alikeness in state among maize inbreds. Theor. Appl. Genet. 93:262-267.

Bohn, M., M. Frisch, and A. E. Melchinger. 2004. A statistical framework for identification of essentially derived varieties obtained from biparental crosses of homozygous lines. I. Statistical theory. In Preparation.

Boppenmaier, J., A. E. Melchinger, G. Seitz, H. H. Geiger, and R. G. Herrmann. 1993. Genetic diversity for RFLPs in European maize inbreds. III. Performance of crosses within versus between heterotic groups for grain traits. Plant Breed. 111:217-226.

Borgo, L., Serani B., and S. Conti. 2002. Molecular profiling of genetically modified maize lines as related to the issue of essential derivation. Poster presented at the Annual Congress of the Italian Society of Agricultural Genetics, Sept 18-21 2002, Girardini Naxos, Italy.

Burstin, J. and A. Charcosset. 1997. Relationship between phenotypic and marker distances: theoretical and experimental investigations. Heredity. 79:477-483.

Dillmann, C., A. Bar-Hen, D. Guerin, A. Charcosset, and A. Murigneux. 1997. Comparison of RFLP and morphological distances between maize (Zea mays L.) inbred lines - consequences for germplasm protection purposes. Theor. Appl. Genet. 95:92-102.

Dillmann, D. and D. Guérin. 1998. Comparison between maize inbred lines: genetic distances in the expert's eye. Agronomie. 18:659-667.

Duvick, D.N. 1984. Genetic contributions to yield gains of USA hybrid maize, 1930 to 1980. p. 15–47. *In* W.R. Fehr (ed.) Genetic contributions to yield gains of five major crop plants. CSSA Spec. Publ. 7. CSSA, Madison, WI.

Foulley, J. L. and W. G. Hill. 1999. On the precision of estimation of genetic distance. Genet. Sel. Evol. 31:457-464.

Frisch, M., M. Bohn, and A. E. Melchinger. 2000. Plabsim: Software for simulation of marker-assisted backcrossing. J. Hered. 91:86-87.

Gethi, J. G., J. A. Labate, K. R. Lamkey, M. E. Smith, and S. Kresovich. 2002. Ssr variation in important us maize inbred lines. Crop Sci. 49:951-957.

Giancola, S., S. Marcucci Poltri, P. Lacaze, and H. E. Hopp. 2002. Feasibility of integration of molecular markers and morphological descriptors in a real case study of a plant variety protection system for soybean. Euphytica. 127:95-113.

Gill, P., C. P. Kimpton, A. Urquhart, N. Oldroyd, E. S. Millican, S. K. Watson, and T. J. Downes. 1995. Automated short tandem repeat (STR) analysis in forensic casework: a strategy for the future. Electrophoresis. 16:1543-1552.

Gilliland, T. J., R. Coll, E. Calsyn, M. de. Loose, M. J. T. v. Eijk, and I. Roldan Ruiz. 2000. Estimating genetic conformity between related ryegrass (*Lolium*) varieties. 1. Morphology and biochemical characterisation. Mol-breed. 6:569-580.

Graham, J., J. Curran, and B. S. Weir. 2000. Conditional Genotypic Probabilities for Microsatellite Loci. Genetics. 155:1973-1980.

Heckenberger, M., M. Bohn, and A. E. Melchinger. 2004. A statistical framework for identification of essentially derived varieties obtained from biparental crosses of homozygous lines. II. SSR data from maize. Crop Sci. submitted.

Heckenberger, M., M. Bohn, J. S. Ziegle, L. K. Joe, J. D. Hauser, M. Hutton, and A. E. Melchinger. 2002. Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties. I. Genetic and technical sources of variation in SSR data. Mol. Breed. 10 :181-191.

Heckenberger, M., J. Rouppe van der Voort, A. E. Melchinger, J. Peleman, and M. Bohn. 2003. Variation of DNA fingerprints among accessions within maize inbred lines and implications for identification of essentially derived varieties. II. Genetic and technical sources of variation in AFLP data and comparison to SSR data. Mol. Breed. 12:97-106.

International Seed Federation. 2002. ISF view on Intellectual Property.

International Seed Federation. 2003. 2002/2003 Progress Report of the Secretariat.

Jaccard, P. 1908. Nouvelles recherches sur la distribution florale. Bull. Soc. Vaud. Sci. Nat. 44:223-270.

Lombard, V., C. P. Baril, P. Dubreuil, F. Blouet, and D. Zhang. 2000. Genetic relationships and fingerprinting of rapeseed cultivars by AFLP: consequences for varietal registration. Crop Sci. 40:1417-1425.

Malécot, G. 1948. Les mathematiques de l'heredité. p. Masson & Cies, Paris.

Marhic, A., S. Anatoine-Michard, J. Bordes, M. Pollacsek, A. Marigneux, and M. Beckert. 1998. Genetic improvement of anther culture response in maize: relationships with molecular, mendelian and agronomic traits. Theor. Appl. Genet. 97:520-525.

Matsuoka, Y., S. E. Mitchell, S. Kresovich, M. Goodman, and J. Doebley. 2002. Microsatellites in Zea - Variability, Patterns of Mutations, and Use for Evolutionary Studies. Theor. Appl. Genet. 104 :436-450.

Melchinger, A.E. 1999. Genetic Diversity and Heterosis. International Symposium on Genetics and Exploitation of Heterosis in Crop Plants. Mexico City. p. 99-118. *In* J.G. Coors and S. Pandey (ed.) The genetics and exploitation of heterosis in crops. ASA, CSSA, and SSSA, Madison, WI.

Melchinger, A. E., M. M. Messmer, M. Lee, W. L. Woodman, and K. R. Lamkey. 1991. Diversity and relationships among U.S. maize inbreds revealed by restriction fragment length polymorphism. Crop Sci. 31:669-678.

Messmer, M. M., A. E. Melchinger, R. G. Herrmann, and J. Boppenmaier. 1993. Relationships among early European maize inbreds. II. Comparison of pedigree and RFLP data. Crop Sci. 33:944-950.

Multani, D. S., S. P. Briggs, M. A. Chamberlin, J. J. Blakeslee, A. S. Murphy, and G. S. Johal. 2003. Loss of an MDR transporter in compact stalks of maize *br2* and sorghum *dw3* mutants. Science. 302:81-84.

Murigneux, A., D. Barloy, P. Leroy, and M. Beckert. 1993. Molecular and morphological evaluation of doubled haploid lines in maize. 1. Homogeneity within DH lines. Theor. Appl. Genet. 86:837-842.

Nuel, G., S. Robin, and Baril C.P. 2001. Predicting distanced using a linear model: the case of varietal distinctness. J. Appl. Stat. 28:607-621.

Rebourg, C., B. Gouesnard, and A. Charcosset . 2001. Large scale molecular analysis of traditional European maize populations. Relationships with morphological variation. Heredity. 86:574-587.

Roldan Ruiz, I., E. Calsyn, T. J. Gilliland, R. Coll, M. J. T. van Eijk, and De LooseM. 2000a. Estimating genetic conformity between related ryegrass (*Lolium*) varieties. 2. AFLP characterization. Mol. Breed. 6:593-602.

Roldan Ruiz, I., F. A. v. Eeuwijk, T. J. Gilliland, P. Dubreuil, C. Dillmann, J. Lallemand, M. de. Loose, and C. P. Baril. 2000b. A comparative study of molecular and morphological methods of describing relationships between perennial ryegrass (*Lolium perenne* L.) varieties. Theor. Appl. Genet. 103:1138-1150.

Smith, J. S. C., E. C. L. Chin, H. Shu, O. S. Smith, S. J. Wall, M. L. Senior, S. E. Mitchell, S. Kresovich, and J. Ziegle. 1997. An evaluation of the utility of SSR loci as molecular markers in maize (*Zea mays* L.) - comparisons with data from RFLPs and pedigree. Theor. Appl. Genet. 95:163-173.

Smith, J. S. C. and O. S. Smith. 1989. The description and assessment of distances between inbred lines of maize: II. The utility of morphological, biochemical, and genetic descriptors and a scheme for testing of distinctiveness between inbred lines. Maydica. 34:151-161.

The Arabidopsis Genome Initiative. 2000. Analysis of the genome sequence of the flowering plant *Arabidopsis thaliana*. Nature. 408:796-815.

Thornsberry, J. M., M. M. Goodman, J. Doebley, S. Kresovich, D. Nielsen, and E. S. Buckler. 2001. *Dwarf8* polymorphisms associate with variation in flowering time. Nat. Genet. 28:286-289.

Troyer, A. F. and T. R. Rocheford. 2002. Germplasm ownership: related corn inbreds. Crop Sci. 42:3-11.

UPOV. 1991. International convention for the protection of new varieties of plants.

Vigouroux, Y., J. S. Jaqueth, Y. Matsuoka, O. S. Smith, W. D. Beavis, J. S. C. Smith, and J. Doebley. 2002. Rate and pattern of mutation at microsatellite loci in maize. Mol. Biol. Evol. 19:1251-1260.

Walsh, B. 2000. Quantitative genetics, genomics, and the future of plant breeding. University of Arizona.

Walsh, B. 2001. Quantitative genetics in the age of genomics. Theor. Pop. Biol. 59:175-184.

Ziegle, J. S., K. P. Corcoran, L. Nie, P. E. Mayrand, L. B. Hoff, L. J. McBride, M. N. Kronick, and S. R. Diehl. 1992. Application of automated DNA sizing technology for genotyping microsatellite loci. Genomics. 14:1026-1031.