STANDER, J.R., Betaseed, Inc. P.O. Box 859, Kimberly, ID 83341. The relationship between biotechnology and classical plant breeding.

Plant breeders have been relatively successful over the years. Duvick estimated that for most grain crops yields have increased continuously since the 1930's. It was estimated that nearly 50% of those gains could be attributed to the enhanced genetic potential of the cultivars. In addition to increased productivity, plant breeders have also been successful in producing remarkable transformations in the quality, growth habits and utilization, as well as the adaptation of certain crops. Whereas these changes have been well documented at the phenotypic level, very little is known about the response to selection at the genotypic level and even less about their biological bases.

In sugarbeets, breeders have likewise made significant advances, but in common with other crops, significant challenges remain. The economics of production are often marginal. Significant losses occur due to fungal and viral diseases, insects, and nematodes.

Plant breeding remains a numbers game in which time is of the essence for growers, industry and consumers. In 1981 Hallauer and Miranda estimated that only one S_2 or S_3 maize line in 10,000 evaluated over the previous 40 years was eventually used to any extent in commercial hybrids. Can biotechnology help us become more efficient? Can we improve these numbers? Where does biotechnology fit in the improvement of crops? Will it change plant breeding, and if so, how?

The results of a survey presented by Phillips in 1983 projected that the US maize yields would continue to increase through the year 2000 largely due to conventional plant breeding and emerging biotechnologies. Contributions from conventional breeding have been realized, while those from biotechnology have lagged behind prediction. This situation does not condemn the biotechnologies; but it does illustrate the difficulty of making predictions about complex biological systems.

Will technology eliminate the need for plant breeders? Frey (1991) stated "Let me assure you that the core of plant breeding will remain very much intact ... The primary sources of genes used in plant breeding will be the primary gene pools of the commodity crops. The primary procedure for deriving new genotypes will be via hybridization and segregation. The most extensive and long term tasks will be field-testing of candidate varieties for zones of adaptation and consumer acceptability." Lande (1991) stated "Experienced plant breeders are well aware that biotechnology can never replace traditional methods of plant breeding, but must be integrated with them to achieve the maximum improvement in crop yield and quality."

It should be remembered that the long term goals of crops biotechnology are the same as those in conventional plant breeding — the creation of improved plant varieties. Moreover, it should be clear that advances made possible through recombinant DNA technology must ultimately be integrated into classical plant breeding programs.

There are several biotechnologies. I shall not address them all, but will restrict my remarks to the technologies of genetic transformation and molecular markers.

GENETIC TRANSFORMATION

In conventional plant breeding, the pool of genes available to the breeder are those genes that exist in plants which are cross-fertile. Genetic transformation essentially obliterates those natural barriers to gene flow. This technology allows us to include genes from other species in the available gene pool.

Herbicide resistance

Herbicide resistance is the earliest application of transformation technology to approach commercialization in several crops including sugarbeets. This technology allows non-selective herbicides such as glufosinate or glyphosate to be applied to sugarbeet.

Disease/Pest resistance

It has been determined that transgenic CP (coat protein) confers viral resistance in many crops. This approach is being evaluated for control of BNYVV and BYV in sugarbeets.

Firms are now advertising the availability of fungal and nematode resistance genes. These genes need to be evaluated. Undoubtedly additional genes conferring viable resistance to many current sugarbeet pests and diseases will be found in the future. I am optimistic that gene transfer technologies will help provide significant assistance in disease and pest resistance.

Conventional resistance breeding efforts are often problematic because of the lack of adequate available resistant source materials, or because of their multigenic inheritance. Transgenic traits which are simply inherited, controlled by single or few genes, will be the best candidates for integration and utilization by breeders.

Some remarkable results were recently reported in Science after the molecular characterization of three resistance genes. The RPS2 gene is from the small mustard-like plant *Arabidopsis thaliana* which provides resistance to a bacterial pathogen — *Pseudomonas syringae*. The N-gene is from tobacco and provides resistance to tobacco mosaic virus (TMV). The L^6 gene is from flax and confers resistance to a fungal rust disease — *Melamspora lini*.

These genes from different species which confer bacterial, viral and fungal resistance, all have common sequence patterns. All code for proteins that have "p loops" (amino acid sequences that bind phosphates of nucleotides and are involved in energetic reactions. And all have leucine rich repeats (amino acid segments which have been associated with protein/protein interactions). Overall, the protein produced by the RPS2 and the N genes have 25% identical and 50% similar sequences.

The implication is, that there may exist underlying mechanisms to disease resistance that could lead to strategies for conferring resistance to a broad variety of pathogens.

In addition to herbicide resistance and disease and pest resistance, many other possibilities exist for modification of sugarbeet production characteristics. It is also possible that the sugarbeets can be used to produce novel polysaccharides or other products, significantly altering the utilization of sugarbeets.

MOLECULAR MARKERS

The suggestion for the utilization of genetic markers to facilitate plant breeding was proposed by Sax over 70 years ago. The basic principle is that selection for characters with easily detectable phenotypes can simplify the recovery of genes of interest linked to them, which may be more difficult to score. The application of this theory since that time has been limited by the lack of available segregating markers.

Recent advances in methods for assaying DNA polymorphisms have produced hundreds of segregating genetic markers in many species. These molecular markers have significant advantages over conventional marker systems.

One of the uses of an RFLP is to "tag" a given gene by locating an RFLP that is tightly linked to the gene of interest. A specific RFLP genotype is then used to mark this gene of interest. The more tightly linked the RFLP and the gene are, the higher the probability is that the presence of one will predict the presence of the other. For example, an RFLP and a disease-resistance gene 1 centimorgan (cM) apart, or with approximately 1% recombination between them, will be separated from each other by a random recombination event only 1% of the time.

Desirable genotypes can be selected using molecular markers rather than scoring for the trait itself. This is important when a trait is recessive, difficult to score, or obscured by other characters. The ability to screen a large breeding population for the RFLP can greatly increase the efficiency of a breeding program in cases where for example in the case of disease resistance, it may be necessary for plants to be grown to maturity. Such markers then could be used for marker assisted selection (MAS) or marker assisted backcrossing (MAB).

<u>Multigenic traits</u>

Probably the most difficult problem facing breeders is the manipulation of metric traits with complex inheritance. Quantitative genetics has been described by Lewontin (1977) as an attempt to produce knowledge through a systemization of ignorance: in most instances, nothing has been known about number and function of genes, linkage, and underlying biology of the trait(s) or process(es) being assessed as reduced statistical entities.

Nevertheless, quantitative genetic principles have fostered the development of useful plant breeding practices such as methodical progeny testing schemes and objective approaches for comparing genetic gain from selection for different breeding schemes.

Markers can be applied to traits with complex inheritance, multigenic traits inherited in a quantitative manner, as well. The application of molecular markers in mapping genes controlling

complex polygenic characters, including those fundamental to crop improvement is particularly valuable. A number of studies have shown RFLP's to be associated with QTL's. Quantitative trait loci (QTL's) cannot be tagged with a single RFLP; however, by examining markers spaced evenly over the genome and correlating specific RFLP genotypes over the population with the measured changes in the QTL, it is possible to mark regions of the genome that contribute to the trait of interest.

Molecular markers provide a mechanism for applying linkage genetic techniques to complex inheritance problems that almost reduces them to the level of studying single gene traits. Among their many applications, one of the more important is the analysis and dissection of complex traits into individual components.

One can determine how many genes are involved in a complex trait, evaluate their gene action and their relative importance, and in some cases relate them to known genes.

Use of markers to understand germplasm relationships — Walton and Helentjaris (1987) working with maize, plotted hybrid yield vs. the dissimilarity of the 2 parents used to create that hybrid. Remarkably, from dissimilarities of about 15% to 60% the relationship was almost linear. One could explain a very high percentage of hybrid yield in corn imply be knowing the RFLP patterns of inbred lines.

Smith and Smith (1989) expanded on this work using more markers and inbred combinations to show that they could account for almost 87% of the hybrid yield over a range of dissimilarities from less than 10 to greater than 90%.

The implication is that knowledge of germplasm relationships can make a breeder more efficient in helping him to sample and utilize available germplasm in a more systematic fashion. It remains to be seen whether similar types of analyses in other crop species will reveal such striking relationships, or if such analyses can be applied in practical breeding.

Quantitatively inherited traits of agricultural significance — Edwards, et al. utilized 114 markers including both isozymes and RFLP's to analyze a set of F_2 plants produced from the cross Co159 X Tx303 detected numerous major loci affecting several metric traits. These factors were relatively localized along the chromosomes and not all regions displayed the same level of impact on these traits.

Using their analysis of height as an example, genetic factors for plant height were not found on all chromosomes nor were they of equal value. In a cross between one very tall inbred and one very short inbred line, factors contributing positively to plant height were contributed by both parents.

Several loci are tabulated that exhibit an effect on plant height. Variation for height can occur through changes in internode length or numbers of nodes. It can be seen that some genes

affecting plant height primarily acted through changing the number of nodes (linked to marker loci NPI_205 and NPI_43) and others through altering the internode distance (linked to marker loci, Adh_2 and Acp_1).

In addition to RFLP'S, there are also now RAPD'S and more recently AFLP'S, microsatellites (SSR's — simple sequence repeats).

Concluding remarks:

Biotechnology offers great promise as a tool to assist the breeder in developing improved cultivars.

1. Gene transfer offers the possibility to broaden the available pool of genes.
2. Molecular marker technology has the potential to make plant breeding more precise, and shorten variety development time.
3. Transformation, RFLP mapping, along with even more sophisticated techniques such as modification of the DNA sequence within genes are powerful tools allowing fundamental studies of how genes work in controlling growth and development. To learn why a particular genotype is tolerant to a disease or to salinity or to drought; can only increase the precision of the breeder.