



ICEBERG LETTUCE

Introduction

Disease resistance, pathogen monitoring, insect resistance, seed quality improvements, resistance to postharvest browning and proprietary line protection are some of the goals of applications of biotechnology to iceberg (crisphead) lettuce improvement. Lettuce breeding and selection have benefited from a variety of novel technologies that are providing new opportunities for both generating and manipulating variation for cultivar improvement. Pesticide applications for virus and fungal disease control will be reduced by a combination of genetic resistance traits and rapid pathogen monitoring, both made possible by applications of biotechnology. Regionally-based identification of shifts in key pathogen race genetics will provide timely guidance for growers to base seed selection and pesticide spray program decisions.

The purpose of this brochure is to provide an overview of the technology and its current and future applications to lettuce.

Overview of Iceberg Lettuce Production in California

California produces over 69% of the iceberg lettuce in the United States. The farm gate value in 1995 was \$986,952,000, representing 4.5% of the total farm products value in California (California Department of Food and Agriculture Statistics Service). Over \$150 million in farm sales are for export markets, of which 85% was shipped to Canada and the remainder to Pacific Rim destinations.



California produces iceberg lettuce year-round. The major production areas for iceberg lettuce in California are Monterey, San Luis Obispo, Santa Barbara, Fresno, and Imperial Counties. For detailed information on lettuce production and pest management, consult DANR Publication #7215 : Iceberg Lettuce Production in California.

Applications of Biotechnology

The application of the tools of biotechnology to iceberg lettuce fall into three general categories: tissue culture techniques for propagation, molecular markers as aids in selection during breeding, and the introduction of transgenes (genetic material introduced by the process of transformation). Although all three have great potential, they merely represent additional sets of

options for lettuce improvement and they will only be effective when integrated with classical breeding procedures.

Tissue culture techniques for propagation:

Lettuce can be readily propagated in culture by a variety of techniques. Propagation from axillary buds (formed at the base of leaves) of heads selected in the field is routinely practiced by some breeding companies. A technique called Embryo Rescue is an important tissue culture technology for more fully accessing the genetic resources found in wild lettuce germplasm. Embryos (a miniature plant within a developing seed) resulting from crosses between *Lactuca* (lettuce) species that would otherwise be nonviable, are “rescued” on culture medium. This tissue culture technique is being used to transfer disease resistance from *Lactuca virosa* and *L. saligna* into cultivated lettuce. Additional technologies utilize isolated, naked plant cells, called protoplasts. Protoplast fusion has been used occasionally to access exotic, sexually incompatible germplasm; however, this has yet to result in commercially useful material.

Molecular Marker Assisted Selection:

Technological advances have produced novel and potentially more efficient ways of analyzing and manipulating natural genetic variation. Several types of genetic markers are now available that differ in their ease of use, cost per test, and type of information they reveal. Using protein-based isozyme markers and DNA-based markers the genetic map of iceberg lettuce is nearing completion. Markers are becoming available for individual resistance genes and certain other traits. However, iceberg lettuce cultivars have a narrow genetic base and the potential for differentiating markers is more limited than with other crops. Novel approaches will be required to provide informative markers throughout the genome. Until such markers are available, it will be difficult to develop molecular markers linked to important horticultural traits.

Selection for Disease Resistance

Molecular markers will play an increasingly important role in breeding for disease resistance in several ways:



Downy Mildew (*Bremia lactucae*) of Lettuce

- (i) Molecular markers allow DNA fingerprinting of wild germplasm and cultivars. Characterization of germplasm using markers linked to resistance genes indicates which germplasm accessions and cultivars carry the same resistance genes. Molecular markers are being used to fingerprint the new sources of resistance to lettuce downy mildew. Testing of seed stocks for identity and purity is already done commercially. Markers linked to resistance genes can also provide diagnostic fingerprints to distinguish a proprietary cultivar.

- (ii) Molecular markers will speed up the introgression of resistance genes from wild species by allowing selection against, and therefore rapid removal of, unwanted chromosomal regions originating from the wild donor parent. Identification of recombination between markers flanking a resistance gene can help break the association of undesirable horticultural traits that may be genetically linked to resistance and would otherwise prevent the use of the resistance gene.
- (iii) It is slow and difficult to select for resistance in lettuce to several diseases, such as bacterial corky root and lettuce mosaic virus. Linked markers will allow indirect selection for resistance genes such as *cor* (*resistance to corky root*) and *mo1* (*resistance to lettuce mosaic virus*). This will allow faster selection at the seedling level and in areas where corky root may not occur.
- (iv) Molecular markers will also allow combinations of resistance genes that would not otherwise be possible. Numerous accessions have now been identified that provide resistance to all isolates of downy mildew; without molecular markers, it is impossible to combine such resistance's from different sources.
- (v) Also, as resistance genes are usually clustered in the genome, molecular markers can be used to identify recombinants that have new combinations of resistance genes on the same chromosome. Once individuals with both resistance genes on the same chromosome have been identified, resistance can be selected using traditional methods.

A current limitation to the application of molecular markers to lettuce improvement is their cost and technical complexity. As markers are identified for more genes, the effort and cost per trait diminishes and therefore it becomes increasingly advantageous to use molecular markers. Also, the technologies for marker analysis are rapidly becoming less expensive and complex. Therefore, marker-aided selection for disease resistance will soon become a routine part of many lettuce breeding programs.

Future Applications

In the longer term, molecular markers will allow the identification of genetic factors determining complex quantitative traits such as field resistance to downy mildew. When such markers have been identified, they will greatly increase the ease with which field resistance can be transferred between lines and combined with other resistance genes.

Molecular markers can also be used to monitor pathogen diversity. They will be particularly useful for monitoring the variation and spread of lettuce downy mildew. Currently, it is slow and laborious to characterize isolates for virulence phenotype, metalaxyl (the primary fungicide for control) sensitivity, and mating type; it is impossible to process isolates fast enough and in sufficient numbers for immediate decisions on disease control. Markers are being developed for diagnostic genes; in the longer-term, markers for metalaxyl sensitivity should come available. Techniques are also being developed for the routine, rapid fingerprinting of hundreds of single-lesion (an individual Downy Mildew infection) isolates. Integration of this information with epidemiological data will allow the targeting of metalaxyl applications to sensitive epidemics and the deployment of cultivars with effective resistance genes. This is becoming increasingly important as the California pathogen population seems to be in transition

from a population in which a few asexual pathotypes predominated to a more variable, sexual population. Currently, one mating type (B₁) is still metalaxyl-sensitive and rare in commercial fields. Application of metalaxyl targeted to sensitive B₁ epidemics could slow the transition to the sexual population.

Transgenic approaches:

Lettuce can be routinely transformed using *Agrobacterium tumefaciens* and appropriate tissue culture conditions (For a description of the process See ABC- Biotechnology Overview). It takes only few weeks longer to go seed to seed via tissue culture than it takes naturally. However, some cultivars remain considerably easier to transform than others. Unfortunately, while it is relatively easy to introduce DNA into lettuce, experience in several labs has shown that transgenes tend to be switched off in later generations. It is unclear how prevalent this is and what mechanism(s) are responsible for this loss of phenotype; however, this phenomenon needs further research before stable expression of transgenes is assured. This is currently a rate-limiting step to the deployment of transgenes in lettuce.

Genes have now been cloned from various species that can confer resistance to viruses, bacteria, fungi, and insects (different genes in each case). In some cases, these technologies may provide the only alternatives to the use of chemical protectants which are environmentally undesirable or facing restrictions on their continued use. Transgenes also offer protection against less-specialized pathogens, such as *Sclerotinia* spp. (the cause of Lettuce Drop), that have been typically difficult to control by traditional breeding. The potential of transgenes is only just beginning to be realized. Examples of the most advanced transgenic strategies are described below.

The success of each strategy in lettuce will depend on a variety of factors. Some of these factors are technical or biological: our ability to identify the genes at the molecular level; the ease with which the transgene can be expressed at suitable levels in the appropriate parts of the plant; any physiological cost to the plant; or potentially detrimental changes to nutritional value (perceived or real). Another limitation will be vulnerability of the agroecosystem if the same transgene (such as *Mi* or *cryIA*, see below) is introduced into multiple crops that are grown in the same area; this will increase the selection for pests or pathogens capable of overcoming the resistance. Other potentially limiting factors are commercial. Transgenic technology is constrained by an evolving web of patents and access to “enabling technologies” and “freedom to operate” in a commercial enterprise. It is unclear how this will impact access and use of transgenes for crops such as lettuce. There is no one disease that will allow a single patented transgene to dominate sales of lettuce seed. The ideal situation would be if transgenes were released into the gene pool for all breeders to use and a royalty paid when seed was sold containing a patented gene. This would allow the full exploitation of transgenic technology and the combining of several transgenes from different sources into a range of horticulturally advanced cultivars. It remains to be seen whether this sensible approach that benefits the grower and consumer can prevail over short-term commercial interests.

Specific Transgenes:

Major genes for disease resistance have been cloned from several plant species. Many of these genes are surprisingly similar at the most basic level of DNA composition. This is aiding in the cloning of additional resistance genes and many more will be cloned from numerous plant species within the next few years. Using a combination of approaches, *Dm* genes for resistance to downy mildew will soon be isolated from lettuce. The cloning and characterization of resistance genes will have a major impact on breeding for disease resistance. It will immediately provide specific molecular markers for selection of resistance genes based on the sequence of the actual genes as described above.

It should be possible to isolate numerous resistance genes from wild species and to introduce them to breeding lines as a cluster of genes. This will allow pyramiding of genes for resistance against multiple isolates of a single pathogen such as downy mildew or against multiple pathogens. This transgenic approach to pyramiding resistance genes overcomes several of the problems associated with pyramiding using traditional backcrossing approaches. Although introduction of multiple genes may lead to the selection of isolates of the pathogen that can overcome all of the new genes, the use of several genes should slow down the appearance of such pathogen genotypes; also, the availability of numerous genes allows several different cassettes to be introduced so that resistance is not dependent on a single set of genes.

Cloned components of pathogens can also be used to provide resistance. Currently the most potent example of this is the expression of parts of viruses, often the coat protein gene. This has been shown to provide high levels of resistance against a wide range of viruses and transgenic cultivars have been approved for commercial use in several crops. All viral diseases of lettuce are potential targets for this strategy and suitable fragments have been cloned from several viral pathogens of lettuce. Constructs containing the coat protein gene of lettuce mosaic virus have been introduced into lettuce and some transgenic plants have high levels of resistance; the stability of expression is currently being determined. Similar experiments have been made with tomato spotted wilt virus and lettuce infectious yellows. However, in these latter cases stable levels of resistance have yet to be obtained (possibly due to problems in obtaining stable expression of the transgenes, see above).

It is also possible to recruit genes from antagonists or parasites of pests and pathogens. The most advanced example of this strategy is the introduction of a *cryIA* gene encoding a delta endotoxin from the bacterium, *Bacillus thuringiensis*, that parasites many insects. It is the active component of several biological pesticides that have been used for many years on lettuce. This protein toxin has been thoroughly studied and has been shown to be harmless to nontarget insects, other animals, and humans. Expression of a *cryIA* gene in a variety of crop plants has resulted in resistance to insects. There is a high degree of specificity between bacterial strains and the insect infected. Endotoxin genes from different strains confer resistance to different insects. Endotoxin genes are available that are effective against several insect pests of lettuce (For more detailed information on *cryIA* and Bt-toxin See ABC -Bt-Potatoes).

It is becoming increasingly possible to manipulate plant morphology, development, and metabolism as more genes are cloned and the regulation of these processes are understood. This will allow the manipulation of several horticultural traits such as plant color, bolting, and

senescence. Biotechnology will also provide the genetic tools to reduce postharvest disorders. Inhibition of genes such polyphenol oxidase has been shown to reduce browning in other crops and has the potential to do the same in lettuce when controlled by suitable regulatory sequences.

Conclusions and Timelines

Biotechnological approaches are already impacting lettuce improvement through in vitro propagation, embryo rescue, and the use of markers for tests of cultivar purity and identity. In the near future, molecular markers will be a routine component of breeding programs as more markers are identified and the technology becomes less expensive and easier to use. The imminent cloning of disease resistance genes from lettuce will impact both classical and transgenic strategies. Several potentially useful transgenes have been introduced into lettuce. However, transgenes seem not to be expressed reliably and therefore it is unclear when transgenic lettuce will be used commercially.

Selected References

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