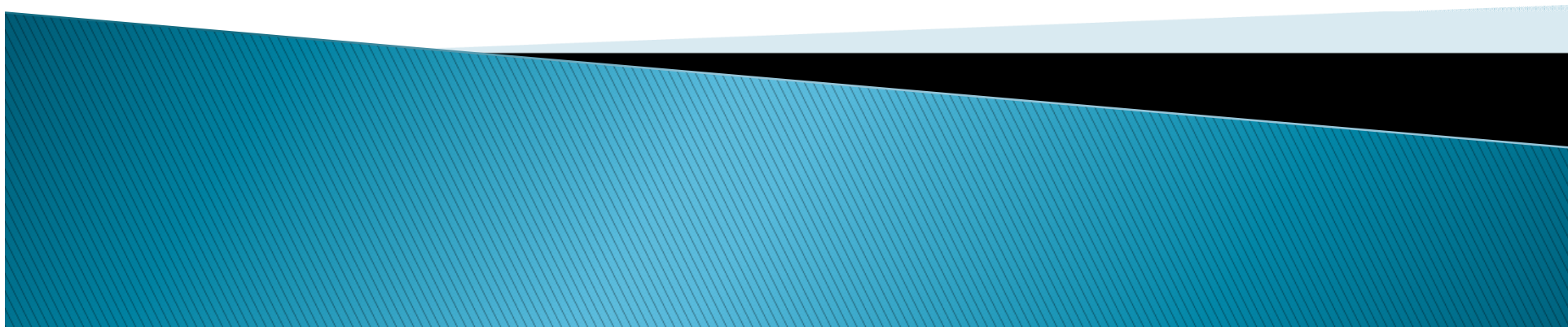


Transformation Needs and Challenges

Lynn Dahleen
USDA-ARS, Fargo, ND
2011 Barley Improvement Conference



Barley transformation–Current status of methods

- ▶ Particle bombardment
 - Efficiencies generally low 1–5%
 - Best cultivar is Golden Promise
 - Can be used with US malting barley cultivars with similar efficiency
- ▶ Agrobacterium–mediated
 - Efficiencies up to 40% in Golden Promise (John Innes)
 - Very low efficiency with US malting barley cultivars
- ▶ Main issue is poor regeneration from transformed cells, specifically germination of somatic embryos



Public acceptance issues

- ▶ Inserting foreign DNA
 - ▶ Potential solution – public and private efforts to design transformation vectors with species-specific DNA
- ▶ Unwanted DNA present with the gene of interest
 - Vector and selectable marker sequence
 - Can be addressed with current technology
- ▶ Lack of control of gene insertion
 - Multiple copies of intact, partial, or rearranged sequences at a locus or at multiple loci
 - Can be addressed with current technology



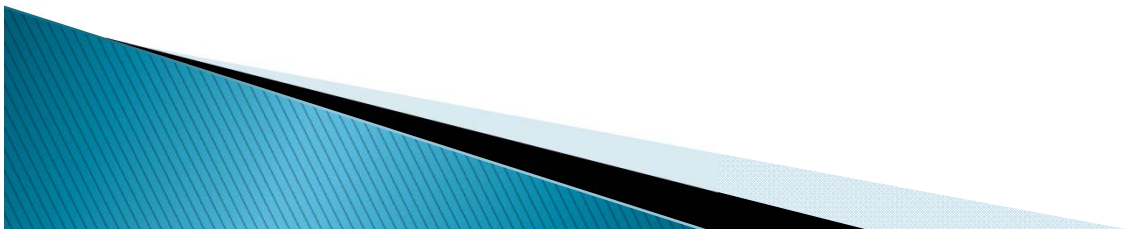
Variety development issues

- ▶ Gene expression
 - Levels of expression
 - Stability of expression
- ▶ Promoter selection
 - An array of promoters for a variety of development times and tissues would be useful
- ▶ Availability of useful genes
- ▶ Knowledge of gene pathways
 - Many important traits involve interactions between multiple genes



Current research to address barley transformation issues

- ▶ Improved regeneration from adapted malting cultivars
- ▶ “Clean gene” systems for marker-free plants
- ▶ Identification of genes useful for barley improvement



Improved regeneration

- ▶ Long-term efforts have improved regeneration from adapted malting cultivars (L Dahleen & P Bregitzer)
 - Conlon transformation a step forward from Golden Promise in the US
 - Looking at newer germplasm, like Pinnacle, Quest
- ▶ Current investigations manipulate hormone levels to increase germination of transformed somatic embryos (Dahleen)
 - Ethylene
 - Cytokinins
 - Auxins
- ▶ Need to limit time in culture to reduce somaclonal variation



Clean gene systems

▶ AC/DS system

- Simplify gene integration patterns by resolving complex loci
- Remove selectable marker genes
- Small numbers of transformants needed to obtain multiple insertion locations in expressed regions
- Increase stability of expression over generations
- System under investigation in barley by P Bregitzer and L Dahleen

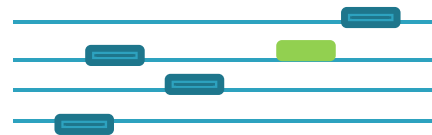


AC/DS system to generate clean transgenics

Create constructs with gene of interest (GOI) and selectable marker gene (SM) flanked by DS inverted repeats

Transformed plant with multiple DS-flanked GOIs and SMs

Transformed plant with AC



Transgenic lines with dispersed DS elements

Self pollinate



Transgenic plants with single DS-flanked GOIs that lack AC and SMs



Clean gene systems

- ▶ Recombinase-mediated cassette exchange
 - Removal of selectable marker genes
 - Site-specific integration
 - Develop an integration platform with recombination site in an expressed chromosome region
 - Use recombinase to insert gene of interest into the recombination site and excise unwanted DNA sequences
 - System under investigation by J. Thomson (ARS, Albany, CA)
 - Advantages over other recombinase systems
 - Unidirectional excision
 - Publically available



Selection of genes for barley improvement

- ▶ Project funded by USWBSI – G Muehlbauer, M Lawton (Rutgers), S Scofield (ARS, West Lafayette, IN)
- ▶ Use microarrays to identify candidate genes important in FHB resistance and DON detoxification
- ▶ Test candidate genes by VIGS in wheat and/or *Physcomitrella* screening
- ▶ Transform wheat and barley with the best candidates

