Protein Production in Plants: Considerations for Platform Choice

Ann Bublitz
BioTech Decisions, Inc.
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Plants Are an Attractive Production Platform

- Key drivers for alternative protein production systems for medicine
  - Growing demand for medicines — increasing and aging population with growing medical needs
  - Cost of developing and producing medicines is increasing
  - Price resistance from consumers and governments
  - If cheaper supply can be produced, demand is likely to be greater
- Opportunities for production of industrial proteins
  - Enzymes for biofuels
  - Other proteins of interest, including feed enzymes, etc.

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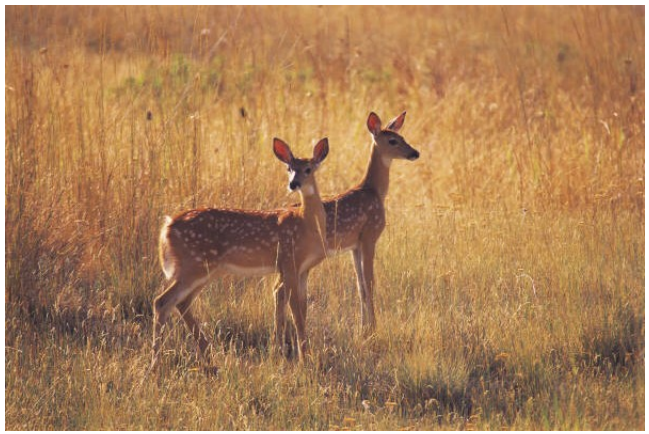
Plants Are an Attractive Production Platform

- Compared to other protein production systems, the use of plant systems to produce raw material tends to:
  - Require considerably reduced capital costs
  - Have lower fixed and operating costs
  - Allow more rapid scale up once a commercial decision to scale up is reached
- However, there are novel risks, especially with field grown plants



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Choice of Plant Platform

- Historically, the plant platform used has been the research focus of the founding company scientist or primary crop of agricultural firm
- Tendency to work with best understood systems, e.g., corn and tobacco
- Limited search for “best” platform; changing platforms is difficult and expensive once work is started
- Must consider properties of the plant, regulatory requirements and IP concerns

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Plant Production Systems

- Field-grown plants
  - Nuclear transformation (most common)
  - Nuclear transformation, expression in organelle (SemBioSys – safflower oil-body)
  - Chloroplast transformation (Chlorogen – tobacco)
- Contained systems
  - Greenhouse (Medicago – alfalfa)
  - Whole plant in enclosed “factory” (Biolex – duckweed)
  - Plant cells in culture (Dow – tobacco)

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Grain vs. Tissue Production Systems

- Advantages of grain
  - Stable protein; typically, long storage time
  - Well understood production systems
- Disadvantages of grain
  - Can take longer time to get to harvestable grain
  - One harvest per season

- Advantages of tissue
  - Faster time to harvestable material
  - Typically, multiple harvest times per season
  - Viable seed production can be avoided, reducing potential for contamination
- Disadvantages of tissue
  - Protein potentially more labile; tissue must usually be processed quickly; adds to cost

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Technical and Production Considerations for Plant Platform

- Technical feasibility
  - Ease of transformation and regeneration of plants
  - Level of protein expression
  - Glycosylation, protein assembly, other post-translational modifications of the system
  - Flexibility of protein production
  - Intellectual property concerns
- Production and economic feasibility
  - Appropriate germplasm base for field production, including sterility control mechanism, if required
  - In-field production costs, during and after production
  - Ease of purification
  - By-product management

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Regulatory Considerations for Plant Platform

- CONTAINMENT!!!
  - Confinement and mitigation conditions adequate?
  - Is it a new or novel crop? New or novel trait?
  - How is the plant pollinated? Bees? Wind? Selfed?
  - Is the plant a food or feed crop?
  - What is the isolation distance to other plants of the same crop?
  - How much area is going to be planted?
  - Is there the potential for cross-breeding with related plants in the planting area?
  - How long do seeds remain dormant and viable in the soil?
  - How persistent is the plant in the environment (tendency to be “weedy”)?

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Regulatory Considerations for Plant Platform

- Environmental Impact*
  - Potential harm to resident or migratory species that are threatened or endangered?
  - Will there be cumulative effects possible with multiple year planting?
  - Are there positive or negative effects on other species? How large are these effects?
- Food/Feed Impact*
  - Allergenicity? Anti-nutritional? Oral toxicant?
  - Is food safety already established? Does the native and plant-produced protein already have GRAS status or is approved as a food additive?

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* Risk assessment results determined more by specific protein than by crop itself, with some exceptions

Containment requirements that add to cost

- Compliance with APHIS regulations (extensive)
- Unauthorized releases and unintended effects must be reported quickly
- Fallow area around plot (50 feet)
- Dedicated planting and harvesting machinery required; equipment cleaning procedures must be provided; dedicated storage facilities required
- Isolation distance from crop to other plantings regulated
- Limitations to crop production in following year(s): monitoring required and land use restrictions
- APHIS inspections required
- Monitoring, notices and extensive reporting during season
- Personnel must be trained by APHIS-approved program
- Approvals required from APHIS for seed handling and other procedures
- State permitting also required

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Source: USDA APHIS

Corn

- Technical Feasibility
  - Transformation ability relatively routine
  - Stable protein storage in grain; medium level of expression
  - Glycosylation occurs; high flexibility in protein production
  - Intellectual property issues relatively well understood
- Production Feasibility
  - Excellent germplasm base available
  - Good economics of production, but will be harder to find acres in Midwest with ethanol demand
  - Ease of purification good if targeted to endosperm
  - More limited by-product with grain
- Containment
  - Wind pollinated
  - Requires 1 mile separation if no fertility control; ½ mile if fertility controlled
  - No wild relatives in most US planting areas
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Both a food and feed crop (primarily feed)
  - Corn itself not a common allergen, anti-nutritional or orally toxic
  - Risk driven by specific protein

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Rice

- Technical Feasibility
  - Transformation ability relatively routine
  - Stable protein storage in grain; high level of expression
  - Glycosylation occurs; high flexibility in protein production
  - Intellectual property issues relatively well understood
- Production Feasibility
  - Very good germplasm base available
  - Difficult to find US acres with food concerns
  - Good economics of production
  - Ease of purification good if targeted to endosperm
  - More limited by-product with grain
- Containment
  - Primarily self-fertilized
  - Relatively lower separation requirement
  - Presence of weedy red rice (relative) must be determined, mitigated and monitored
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Primarily a food crop
  - Rice itself not a common allergen, anti-nutritional or orally toxic
  - Risk driven by specific protein

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Barley

- Technical Feasibility
  - Transformation ability somewhat routine
  - Stable protein storage in grain; potentially high level of expression
  - Glycosylation occurs; potentially high level of flexibility in protein production
  - Intellectual property issues relatively well understood
- Production Feasibility
  - Very good germplasm base available
  - Good economics of production
  - Ease of purification likely to be good
  - More limited by-product with grain
- Containment
  - Primarily self-fertilized
  - Relatively lower separation requirement
  - No cross-hybridization with other plants in US planting areas
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Both a food and feed crop
  - Barley itself not a common allergen, anti-nutritional or orally toxic
  - Risk driven by specific protein

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Alfalfa

- Technical Feasibility
  - Transformation and regeneration relatively routine
  - Protein production in leaf tissue; medium level of expression
  - Glycosylation occurs; reduced stability of leaf tissue limits protein production flexibility
  - Some IP issues
- Production Feasibility
  - Good germplasm base available
  - Good economics of production
  - Purification more difficult with tissue-based production
  - More by-product with tissue based production
- Containment
  - Insect pollinated
  - Requires 1 mile separation if no fertility control; ½ mile if fertility controlled
  - No wild relatives in most US planting areas
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Both a food and feed crop (primarily feed)
  - Alfalfa itself not a common allergen, anti-nutritional or orally toxic
  - Risk driven by specific protein

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Soybeans

- Technical Feasibility
  - Transformation and regeneration still not routine
  - Stable protein storage in grain; medium level of expression
  - Glycosylation occurs; less flexibility in protein production than some other grains
  - Some IP issues
- Production Feasibility
  - Very good germplasm base available
  - Good economics of production
  - Ease of purification will depend upon nature of protein product
  - More limited by-product with grain
- Containment
  - Primarily self-pollinated
  - No wild relatives in US planting areas
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Both a food and feed crop (primarily feed)
  - Soy itself is a common allergen and may be anti-nutritional
  - Risk driven by specific protein

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Safflower

- Technical Feasibility
  - Transformation ability somewhat routine
  - Stable protein storage in grain; medium level of expression
  - Little or no glycosylation with oilbody production system; reduces flexibility of protein production
  - Some IP issues
- Production Feasibility
  - Good germplasm base available
  - Good economics of production
  - Ease of purification very good with oil-body system
  - More limited by-product with oil-body production system

- Containment
  - Primarily self-fertilized
  - Relatively easy to separate from other safflower plantings; 2 miles in permit
  - No wild relatives in most US planting areas
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
  - Monitoring required for following 2 years for volunteers and compatible species
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Both a food and feed crop (primarily grown for edible oil)
  - Safflower oil itself not a common allergen, anti-nutritional or orally toxic; oilbody system involves oleosin which has low probability of being allergen
  - Risk driven by specific protein

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Tobacco

- Technical Feasibility
  - Transformation ability routine
  - Protein production in leaf tissue; medium level of expression
  - Glycosylation occurs with nuclear transformation; no glycosylation with chloroplast transformation, reducing flexibility of protein production
  - Some IP issues
- Production Feasibility
  - Fair germplasm base available
  - Tobacco is a more expensive crop to grow
  - Purification more difficult with tissue-based production
  - More by-product with tissue based production
- Containment
  - Seed production typically prevented; chloroplast transformation reduces dissemination by seed
  - Minimum ¼ mile isolation distance
  - No wild relatives in most US planting areas
  - Seed dormancy in soil less than 2 years
  - Crop does not persist without intervention
- Environmental impact: Driven by specific protein
- Food/feed impact
  - Not a food or feed crop; non-target species unlikely to feed
  - Food safety generally not established
  - Risk driven by specific protein

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Plant Platform Comparison

	Technical feasibility	Production feasibility	Containment & food/feed impact	Overall Ranking
Corn	High	Very high	Medium	3
Rice	Very high	High	High	1
Barley	High	High	High	2
Alfalfa	Medium	Medium	Medium	6
Soybean	Medium	High	Medium	5
Safflower	Medium	Very high	Medium-high	4
Tobacco	Medium	Low-medium	Medium-high	7

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But there is no perfect platform!

Uncertainties

- Regulatory and IP Issues
  - Development and delivery of cGMP protocols throughout production
  - Regulation of follow-on biologics
  - Obtaining IP freedom to operate
- Market acceptance
  - To date, not embraced by pharmaceutical industry
  - Pricing of PMPs may not be low enough to compete with other biopharmaceutical production systems
  - Finding industrial proteins that can justify as PMP
- Protein expression levels and purification efficiencies
  - Not as high as hoped
  - Depends upon protein and crop platform
- Finding experienced growers
  - Development companies underestimate complexity of field production
  - Relatively few farmers familiar with production
  - Failures in growing have nearly “killed” PMPs in the past
- Food industry acceptance
  - “StarLink” experience has made food industry very concerned about PMPs in the food supply
  - Required to test for StarLink protein for years after introduction
  - Want “zero tolerance” for contamination kept in place
- Consumer acceptance
  - US customers relatively accepting of GMOs in general; European not
  - Concerned about more “accidents”
  - Important for benefits to be demonstrated (new, cheaper medicines or fuels) that outweigh the risks

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