Chapter 2
Commercial Plant-Produced Recombinant Avidin

Elizabeth E. Hood and John A. Howard

2.1 Introduction to the Protein Product

Chicken egg white avidin was the first recombinant protein product manufactured for sale in a transgenic plant. Prior to its commercialization, there were many questions as to the validity of using plants as a platform to produce recombinant proteins: doubts were raised as to the ability of plants to express animal or microbial proteins, the ability to obtain proper processing and glycosylation, and the ability to extract and purify these proteins in an economical manner. Therefore, while avidin has modest economic value, it served as the model to launch this approach for a number of other recombinant proteins.

Avidin (C.A.S.: 1405-69-2) is a glycoprotein found in avian, reptilian, and amphibian eggs and is used commercially as a diagnostic reagent. It was first isolated from chicken egg white and named "avidin" in the 1940s (Thompson et al. 1941). The protein avidin comprises four identical subunits, each 128 amino acids long, the amino acid sequence of which was published in 1971 (DeLange and Huang 1971). The carbohydrate moiety is composed of four glucosamine and five mannose residues and is attached to Asn-17 of each subunit (DeLange and Huang 1971). The cDNA of the chicken oviduct *avidin* gene was identified (Gope et al. 1987) and a genomic clone was isolated (Keinanen et al. 1988). They (Keinanen et al. 1988) also reported on a family of closely related *avidin* genes from chicken.

Avidin binds the vitamin biotin with high affinity. Each of the four subunits in the homotetramer binds one biotin molecule. The dissociation constant of the avidin–biotin complex was determined to be 10^{-15} (Green 1963), exhibiting the

E.E. Hood (✉)
Arkansas Biosciences Institute, Arkansas State University, Jonesboro, AR 72467, USA
e-mail: ehood@astate.edu

J.A. Howard
Applied Biotechnology Institute, CalPoly Technology Park, San Luis Obispo, CA 93407, USA
e-mail: jhoward@appliedbiotech.org

J.A. Howard and E.E. Hood (eds.), *Commercial Plant-Produced Recombinant Protein Products*, Biotechnology in Agriculture and Forestry 68,
DOI 10.1007/978-3-662-43836-7_2,

highest known affinity in nature between a ligand and a protein (Livnah et al. 1993). The binding of avidin to biotin is responsible for its commercial value, since it allows for detection of proteins and nucleic acid molecules incorporating biotin. Avidin or avidin subunits can also be used for affinity purification of biotinylated molecules (Berger and Wood 1975; Green and Toms 1973). In nature biotin functions as a cofactor with many enzymes in vivo. Because avidin binds strongly to biotin, it can act as a defense agent against microbial pathogens that are sensitive to biotin levels (Wallen et al. 1995). A second biotin-binding protein is bacterial streptavidin. Although these two proteins show similar activity and tertiary structure, their amino acid sequences are only 30 % identical and are likely not derived from the same ancestral source (Laitinen et al. 2006).

Scientists at Pioneer Hi-Bred International noticed that avidin could inhibit growth in some insects by interfering with their digestion. Transgenic maize plants expressing the chicken *avidin* gene were generated to test it as an insecticidal reagent incorporated into maize leaves and roots (Hood et al. 1997). This observation was later followed up and shown to be very effective to prevent postharvest insect damage while not interfering with metabolism in mammals (Kramer et al. 2000). The transgenic maize plants had a secondary phenotype in that they could confer male sterility and have been suggested as a containment mechanism for transgenic traits in the field (Albertsen et al. 1999).

The primary source for commercial production of avidin is chicken egg white, although the recombinant form is also available (Sigma Chemical Co. A8706). The manufacture of purified avidin protein using a plant source as an alternative to eggs provides benefits such as the absence of animal viruses. Plant-produced avidin provided answers to many of the basic questions about plant-expressed proteins and what is critical for commercialization, providing conditions that are in use today.

2.2 Description of the Systems Used to Produce the Protein

2.2.1 Theoretical Advantages of the Plant Process over Other Technologies

Avidin is usually purified from egg whites (http://www.mastbio.co.kr/root/product/life/ps/gradiflow/pdf/MB-10-Puri-HighlyBasicProteinsAvidinandLysozyme.pdf), where it is present at a concentration of approximately 1.5 mg per egg. More recently, biologically active recombinant isoforms have been produced in several expression systems, including *Escherichia coli* (Airenne et al. 1994), *Picchia* (Zocchi et al. 2003), and baculovirus-infected cells (Airenne et al. 1997). A huge number of variants of avidin have been produced that have applications in various diagnostic and purification kits (Laitinen et al. 2006).

The advantages of a plant recombinant system over the others currently used are that: (1) scale-up is more economical in a plant system due to less expensive substrates (corn grain versus eggs) and greater biomass availability, (2) co-purification of animal pathogens is avoided in a plant system, and (3) if expression is directed to seed, it provides a natural storage system for long duration without degradation.

2.2.2 Past Efforts in Plants

A number of laboratories have experimented with expressing avidin in plants, primarily for its insecticidal properties (Murray et al. 2002; Lichtfouse et al. 2010; Burgess et al. 2002; Markwick et al. 2003; Murray et al. 2010; Masarik et al. 2003). In many cases, the transgenic plants were insect resistant reaching the goal of the project.

2.2.3 Bench Marks of What Is/Was Needed to Commercialize the Product in This System

Most of the initial work with avidin expression in different plants was not designed to overproduce the protein for purification and sale. The maize seed production system, on the other hand, was suitable for the production of the protein for sale as a purified or partially purified product primarily for use in diagnostic kits. High-level expression is required for cost-effective production in the plant system to meet commercial targets. Assuming that the competitive production system is from egg whites, one dozen eggs would produce about 18 mg of avidin for a cost of about $2 for the raw materials. Eighteen mg of recombinant protein from corn seed expressing the protein at 1 % of total soluble protein would require approximately 200 g of grain. At today's high price of $7 per bushel (25 kg), this grain would cost ~$0.06. One percent of TSP has been achieved for multiple proteins in corn seed, and avidin levels as high as 40–50 % of TSP in some selected lines have been obtained. Clearly, the corn system offers economic advantages over the egg system as it relates to the cost of raw materials. In addition, higher concentration of avidin in the biomass leads to a lower cost of purification.

Because proteins produced from plants were new to the market, quality assessment of the product had to be performed to understand the impurities in the product and to build a certificate of analysis, a quality control protocol, and a Material Safety Data Sheet (MSDS) for the product. Each of these was developed for this new product for Sigma Aldrich Chemical Co., which is still the vendor for the product. Characteristics of the protein and product are described below.

2.3 Technical Progress

2.3.1 What Was Achieved?

Many technical tools that were sought after in the mid-1990s are the same today for expression of foreign genes in plants. These include use of a strong promoter, use of an intron particularly for monocot expression, recognition of the need for codon usage that is compatible with the host species, avoidance of toxicity, and targeting the protein to specific subcellular locations that induce maximum expression of the protein (Streatfield 2007). Indeed, each of these molecular parameters was utilized for avidin.

Avidin in maize seed was first produced over 16 years ago (Hood et al. 1997). The molecular technology available at the time was much less sophisticated than technology available today. The gene was synthesized with maize codon usage bias and fused with the barley alpha amylase signal sequence (BAASS) (Rogers 1985), also synthesized with maize codons. Each of the genes/fragments was synthesized as short, overlapping, complementary oligonucleotides with restriction enzyme sites engineered onto the ends and ligated after digestion. All movement between cloning vectors was done with restriction enzyme digestion and ligation. The expression cassette with the constitutive maize ubiquitin promoter (Christensen et al. 1992) and the *pinII* terminator (An et al. 1989) was built separately from the herbicide selection vector for co-bombardment of maize callus tissue. Selection was on the herbicide, bialaphos, using the *bar* gene (White et al. 1990) driven by the CaMV 35S promoter. At that time, biolistic transformation was the most efficient way to introduce genes into corn (USP#5,489,520).

2.3.2 What Expected or Unexpected Hurdles Were Overcome to Reach the Target?

Transgenic events that were resistant to bialaphos and contained the avidin gene as identified by PCR were recovered from transformations. Plants were regenerated from these events; they produced ears in a greenhouse and were pollinated with a proprietary inbred line (Pioneer Hi-Bred PHN46). The highest expressing event was screened by DNA blot hybridization for copy number and insertion sites (Hood et al. 1997). It appeared that three to five insertions were present in this event for both the *avidin* and *bar* genes. When T1 seed was planted for seed increases, the T2 generation plants were no longer resistant to the herbicide. Thus, another method of screening for the segregating (transgenic versus non-transgenic) plants was required. Initially, PCR was performed to track the presence of the *avidin* gene. Observations of the plants in the field revealed that male sterility was present among them at a high percentage. When the PCR results were compared to the

Table 2.1 Increases in inbred germplasm and avidin during several back cross generations of breeding and selection [derived from Hood (2004)]

Year	Generation of see post tissue culture	% Inbred germplasm	Avidin as % DW of grain
1995	T1	50	0.01
1996	T2	75	0.02
1997	T3	87.5	0.05
1998	T4	93.75	0.1
1999	T5	96.88	0.3
2000	T6	98.44	0.7
2001	T7	99.2	1.0

male sterility phenotype, it was discovered that these traits co-segregated to a high degree (97.5 %). Thus, in future generations, transgenic plants expressing the avidin gene were selected by their male sterile phenotype. Although this phenotype is useful for sequestration of the transgenic trait in the environment, it inhibits the ability to recover self-pollinated, homozygous lines.

The Hi-II tissue culture genotype (Armstrong et al. 1991) does not produce well in the field and is highly susceptible to insects and pathogens. Thus, when transformation is performed with the Hi-II line, the resulting events must be back-crossed into elite inbred lines to allow production lines to be established. During back-crossing, it was discovered that increases in transgene-encoded protein could also be recovered in addition to improved agronomic characteristics (Hood et al. 2012). Prior to this point, it was assumed by most scientists that the expression level in T1 seeds would be the same for future generations. However, for maize, introgression into elite lines with selections for expression at each generation led to much higher levels of target protein accumulation. This initial observation has held true for all foreign proteins that we have expressed in corn although they accumulate in seed to greater or lesser amounts (Streatfield et al. 2002; Woodard et al. 2003; Hood et al. 2003, 2012). For avidin, we recovered up to 1 % of dry weight of grain in later generations (Table 2.1) (Hood 2004). Some of this improvement is due to improved germplasm and some is due to more stringent selection of transgenic lines using the male sterility trait to prevent mixing of avidin and non-avidin plants. However, the mechanism(s) driving the improvement in accumulation is unknown. Modern genomic and transcriptomic techniques however should allow a more satisfactory explanation in the near future (Teoh et al. 2013).

The corn-produced avidin was functionally equivalent to the egg-derived protein (Table 2.2). The only obvious physical difference was that the corn protein had slightly less glycosylation—the deglycosylated maize-derived proteins showed the same molecular weight as the native apoprotein (Hood et al. 1997). Binding of the complex protein to biotin, the N-terminal protein sequence, and the pI was the same for protein from either source. These characteristics allow its direct substitution into assay kits from a functionality standpoint.

Because avidin was one of the first highly expressed plant-produced proteins, it was used for many demonstration and pilot studies. One such study was to discover the characteristics of a protein fed to animals (Fig. 2.1). Were antibodies produced?

Table 2.2 Comparison of biochemical properties of native egg avidin and corn-derived recombinant avidin

Biochemical properties	Egg white avidin	Recombinant avidin
Binding stoichiometry	Binds one biotin per subunit	Binds one biotin per subunit
pI	10	10
KI	3.16 μM	3.34 μM
Antigenic similarity	Identical	Identical
Glycosylated	Yes	Yes
N-terminal sequence	Identical	Identical
Molecular weight apoprotein	12,500 kDa	12,500 kDa

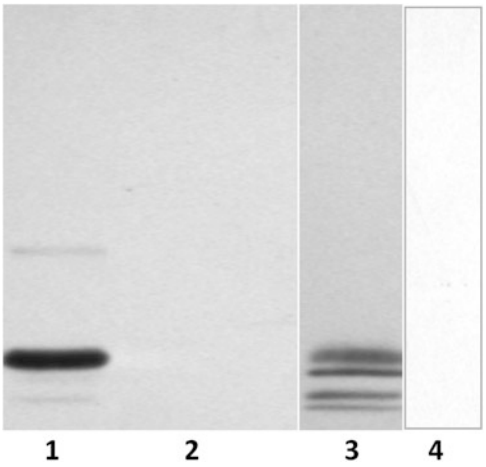

Fig. 2.1 Western blot of feces extracts from mice fed various diets. Total protein was separated on a 12 % SDS polyacrylamide gel and proteins were blotted onto a PVDF membrane (Millipore). Detection of avidin was with an anti-avidin antibody and a secondary antibody conjugated to alkaline phosphatase (AP). AP was detected with a chemiluminescent substrate. Lanes: 1: 50 mg standard avidin; 2: control corn diet; 3: avidin corn diet; 4: mouse chow with 50 mg pure avidin added

If so, what types of antibodies were they? What was the fate of the protein after being fed to animals? These, and other questions, were addressed in a Master of Science thesis study at Texas A&M University (Bailey 2000).

"Avidin corn meal was successfully used to stimulate both serum and mucosal immune responses when fed as the sole diet of mice for several days" (Bailey 2000). In these early studies, seven doses of formulated corn meal were used to stimulate a mucosal response, while nine doses were required for a serum response. Corn was fed as the sole diet, and the doses administered through various feeding regimens of corn meal plus or minus avidin. One of the most interesting outcomes of this study was that the protein survived in the gut after ingestion only when the protein was encapsulated into the grain matrix. Doses of control avidin that comprised purified protein added to the mouse diet were completely degraded in

the gut. This outcome has implications for the quality of antigen presentation to the immune system when fed orally to an animal and was the basis to explore orally administered vaccines (discussed in later chapters in this book).

2.4 Nontechnical Hurdles

2.4.1 Production, Regulatory, Public Perception, Intellectual Property

Although avidin is used in multiple diagnostic products for multiple markets, the production volumes are relatively small: in the order of hundreds of kilograms per year, rather than tons. At the average concentration of 1.5 mg of avidin per egg, a dozen eggs would only produce 18 mg, an amount available from 3.5 g of corn (Fig. 2.2). Indeed, over 800 kg of eggs would be required to produce 20 g of avidin (Hood et al. 1997). Currently, the concentration of avidin in corn seed is up to an average of approximately 0.5 % of dry weight; thus, 4 kg of grain would be required to yield the equivalent 20 g of avidin. Corn grain weighs 25 kg per bushel, and 20 g of avidin could be produced from about 1/6 of a bushel of corn. At 2013 prices, that would be about $1 worth of corn. Even if one triples the price for small volumes and growth under permit, the cost would only be about $3 for the raw materials for protein production. Clearly, this is an advantage over production costs of eggs.

In addition to the cost of raw material, the processing to a highly purified product is usually the most expensive part of the final product. One of the most critical cost factors in this regard is the concentration of the protein in the biomass that is to be used in extraction. Higher concentrations lead to lower unit costs of extraction and usually require less effort in purification as well. In this case, >tenfold higher concentrations in the initial maize biomass can drastically reduce downstream purification cost. Furthermore, as this protein accumulates predominantly in the germ of the kernel, the routine operation of separating the germ from the endosperm as performed in dry milling operations can result in another tenfold reduction in biomass and further reduce downstream cost. Cost models have been created using these factors that have been discussed previously (Howard et al. 2011). An additional advantage is the long-term stability of the product in the grain allowing storage of the raw material for months to years (Kusnadi et al. 1998).

The male sterility trait of the avidin corn does not allow for making homozygous lines. Thus, the trait segregates at every generation, forcing selection of the transgenics from the null segregants for production. Loss of the herbicide resistance trait early on makes production challenging because selection of the transgenic plants cannot be done with the herbicide. Thus, visual scoring is now the only technique with which selection can be done, forcing labor-intensive selection. Fortunately, the amount of protein required for production is low, and this type of production scheme is not prohibitively expensive. One acre of avidin at 0.5 % of dry weight

Fig. 2.2 Avidin from Sigma Chemical Co. purified from corn grain. The number of eggs shown would be required to purify an amount of avidin present in the grain shown

and 160 bushels per acre would yield 20 kg of protein, easily within the production quantities required.

Production of transgenic seed crops for reagent chemicals is done under permit because of the small volume market opportunity. Thus, nonregulated status was not sought and likely will not be necessary to make a profit on the products. These product volumes do not impact the food versus nonfood debate of using plants for purposes other than for food because of their small volume production. At 20 kg per acre, the entire annual product volume of avidin would require fewer than 100 acres. When considering the total volume of corn production, i.e., approximately 100 million acres, then 100 acres for a specialty product have no significant impact.

Even using the best containment precautions, there is always the concern that inadvertent exposure could occur due to some unforeseen event. This is usually thought of as unwanted pollination. In this case, however, this is highly unlikely since the recombinant avidin causes male sterility. Pollen drift is not the only method of exposure, however, and like all other non-plant production systems, contamination of the food chain in any number of ways is considered when setting containment guidelines. The maize production system offers another safeguard in that corn is cooked prior to human consumption which will completely inactivate avidin. This is most evident in that we consume eggs routinely without any adverse effect as long as they are cooked.

2.5 Conclusions

Twenty years ago, the concept of proteins produced in plants was novel. The early successes were important to demonstrate that the technology works. Production of avidin in transgenic maize fulfilled that demonstration. Plant-produced avidin was also used to demonstrate that orally fed proteins could induce circulating and mucosal antibodies in mice. The fact that small amounts of the fed protein, in this

case avidin, survived the gut was an unexpected discovery, but helped to explain why other orally fed purified proteins were not successful vaccines—the protein had to be part of dietary fiber to be protected long enough in the gut to induce a response. The corn containing avidin proved to be resistant to grain storage insects (Kramer et al. 2000). Of additional interest, however, is the male sterile phenotype induced by the avidin gene expressed from the constitutive ubiquitin promoter. Avidin as the first plant-produced protein sold set the stage and debunked pessimism about plant-produced proteins.

2.6 Future Directions

The worldwide market for avidin as a component of diagnostic kits is in the kilogram range. Thus, using the corn seed production system, this market could easily be filled from a few acres of production. More lucrative applications of avidin would be as an inducer of male sterility or insect resistance. These latter uses of the avidin trait would have utility in agriculture, but would require development by a seed company. Continued reagent sales and potential application in diagnostic kits are the most likely market outcomes.

References

Airenne KJ, Sarkkinen P, Punnonen E-L, Kulomaa MS (1994) Production of recombinant avidin in Escherichia coli. Gene 144(1):75–80, http://dx.doi.org/10.1016/0378-1119(94)90206-2

Airenne KJ, Oker-Blom C, Marjomäki VS, Bayer EA, Wilchek M, Kulomaa MS (1997) Production of biologically active recombinant avidin in baculovirus-infected insect cells. Protein Expr Purif 9(1):100–108, http://dx.doi.org/10.1006/prep.1996.0660

Albertsen MC, Howard JA, Maddock S (1999) Induction of male sterility in plants by expression of high levels of avidin. USA Patent

An G, Mitra A, Choi HK, Costa MA, An K, Thornburg RW, Ryan CA (1989) Functional analysis of the 3[prime] control region of the potato wound-inducible proteinase inhibitor II gene. Plant Cell 1(1):115–122. doi:10.1105/tpc.1.1.115

Armstrong C, Green C, Phillips R (1991) Development and availability of germplasm with high type II culture formation response. Maize Genet Coop News Lett 65:92–93

Bailey M (2000) A model system for edible vaccination using recombinant avidin produced in corn seed. Texas A&M University, College Station, TX

Berger M, Wood HG (1975) Purification of the subunits of transcarboxylase by affinity chromatography on avidin-sepharose. J Biol Chem 250(3):927–933

Burgess EJ, Malone L, Christeller J, Lester M, Murray C, Philip B, Phung M, Tregidga E (2002) Avidin expressed in transgenic tobacco leaves confers resistance to two noctuid pests, helicoverpa armigera and spodoptera litura. Transgenic Res 11(2):185–198

Christensen A, Sharrock R, Quail P (1992) Maize polyubiquitin genes: structure, thermal perturbation of expression and transcript splicing, and promoter activity following transfer to protoplasts by electroporation. Plant Mol Biol 18:810–812

DeLange RJ, Huang TS (1971) Egg white avidin. 3. Sequence of the 78-residue middle cyanogen bromide peptide. Complete amino acid sequence of the protein subunit. J Biol Chem 246 (3):698–709

Gope ML, Keinanen RA, Kristo PA, Conneely OM, Beattie WG, Zarucki-Schulz T, O'Malley BW, Kulomaa MS (1987) Molecular cloning of the chicken avidin cDNA. Nucleic Acids Res 15(8):3595–3606

Green NM (1963) Avidin. 3. The nature of the biotin-binding site. Biochem J 89:599–609

Green NM, Toms EJ (1973) The properties of subunits of avidin coupled to sepharose. Biochem J 133(4):687–700

Hood EE (2004) Bioindustrial and biopharmaceutical products from plants. In: New directions for a diverse planet: proceedings for the 4th international crop science congress, 26 September–1 October 2004, The Regional Institute Ltd, Brisbane, Australia

Hood E, Witcher D, Maddock S, Meyer T, Baszczynski C et al (1997) Commercial production of avidin from transgenic maize: characterization of transformant, production, processing, extracting, and purification. Mol Breed 3:291–306

Hood EE, Bailey MR, Beifuss K, Magallanes-Lundback M, Horn ME, Callaway E, Drees C, Delaney DE, Clough R, Howard JA (2003) Criteria for high-level expression of a fungal laccase gene in transgenic maize. Plant Biotechnol J 1(2):129–140. doi:10.1046/j.1467-7652.2003.00014.x, PBI014 [pii]

Hood EE, Devaiah SP, Fake G, Egelkrout E, Teoh K, Requesens DV, Hayden C, Hood KR, Pappu KM, Carroll J, Howard JA (2012) Manipulating corn germplasm to increase recombinant protein accumulation. Plant Biotechnol J 10:20–30. doi:10.1111/j.1467-7652.2011.00627.x

Howard JA, Nikolov Z, Hood EE (2011) Enzyme production systems for biomass conversion. In: Hood EE, Nelson P, Powell R (eds) Plant biomass conversion. Wiley, Ames, IA, pp 227–253, http://dx.doi.org/10.1002/9780470959138.ch10

Keinanen RA, Laukkanen ML, Kulomaa MS (1988) Molecular cloning of three structurally related genes for chicken avidin. J Steroid Biochem 30(1–6):17–21

Kramer K, Morgan T, Throne J, Dowell F, Bailey M, Howard J (2000) Transgenic avidin maize is resistant to storage insect pests. Nat Biotechnol 18:670–674

Kusnadi AR, Hood EE, Witcher DR, Howard JA, Nikolov ZL (1998) Production and purification of two recombinant proteins from transgenic corn. Biotechnol Prog 14(1):149–155

Laitinen OH, Hytonen VP, Nordlund HR, Kulomaa MS (2006) Genetically engineered avidins and streptavidins. Cell Mol Life Sci 63(24):2992–3017. doi:10.1007/s00018-006-6288-z

Lichtfouse E, Martin H, Burgess EJ, Masarik M, Kramer K, Beklova M, Adam V, Kizek R (2010) Avidin and plant biotechnology to control pests. In: Engineering G (ed) Biofertilisation, soil quality and organic farming, vol 4, Sustainable agriculture reviews. Springer, Netherlands, pp 1–21

Livnah O, Bayer EA, Wilchek M, Sussman JL (1993) Three-dimensional structures of avidin and the avidin-biotin complex. Proc Natl Acad Sci USA 90(11):5076–5080

Markwick N, Docherty L, Phung M, Lester M, Murray C, Yao J-L, Mitra D, Cohen D, Beuning L, Kutty-Amma S, Christeller J (2003) Transgenic tobacco and apple plants expressing biotin-binding proteins are resistant to two cosmopolitan insect pests, potato tuber moth and lightbrown apple moth, respectively. Transgenic Res 12(6):671–681

Masarik M, Kizek R, Kramer K, Billova S, Brazdova M, Vacek J, Baley M, Jelen F, Howard J (2003) Application of avidin-biotin technology transfer stripping square-wave voltammetry for detection of DNA hybridization and avidin in transgenic avidin maize. Anal Chem 75:2663–2669

Murray C, Sutherland P, Phung M, Lester M, Marshall R, Christeller J (2002) Expression of biotin-binding proteins, avidin and streptavidin, in plant tissues using plant vacuolar targeting sequences. Transgenic Res 11(2):199–214

Murray C, Markwick N, Kaji R, Poulton J, Martin H, Christeller J (2010) Expression of various biotin-binding proteins in transgenic tobacco confers resistance to potato tuber moth, Phthorimaea operculella (Zeller) (fam. Gelechiidae). Transgenic Res 19(6):1041–1051

Rogers JC (1985) Two barley alpha-amylase gene families are regulated differently in aleurone cells. J Biol Chem 260(6):3731–3738

Streatfield S (2007) Approaches to achieve high-level heterologous protein production in plants. Plant Biotechnol J 5:2–15

Streatfield S, Mayor J, Barker D, Brooks C, Lamphear B, Woodard S, Beifuss K, Vicuna D, Massey L, Horn M, Delaney D, Nikolov Z, Hood E, Jilka J, Howard J (2002) Development of an edible subunit vaccine in corn against enterotoxigenic strains of Escherichia coli. In Vitro Cell Dev Biol Plant 38(1):11–17. doi:10.1079/ivp2001247

Teoh KT, Requesens DV, Devaiah SP, Johnson D, Huang X, Howard JA, Hood EE (2013) Transcriptome analysis of embryo maturation in maize. BMC Plant Biol 13(1):19

Thompson RC, Eakin RE, Williams RJ (1941) The extraction of biotin from tissues. Science 94 (2451):589–590. doi:10.1126/science.94.2451.589

Wallen MJ, Laukkanen MO, Kulomaa MS (1995) Cloning and sequencing of the chicken egg-white avidin-encoding gene and its relationship with the avidin-related genes Avr1-Avr5. Gene 161(2):205–209

White J, Chang SY, Bibb MJ, Bibb MJ (1990) A cassette containing the bar gene of Streptomyces hygroscopicus: a selectable marker for plant transformation. Nucleic Acids Res 18:1062

Woodard S, Mayor J, Bailey M, Barker D, Love R, Lane J, Delaney D, McComas-Wagner J, Mallubhotla H, Hood E (2003) Maize (Zea mays)-derived bovine trypsin: characterization of the first large-scale, commercial protein product from transgenic plants. Biotechnol Appl Biochem 38(2):123–130

Zocchi A, Marya Jobé A, Neuhaus J-M, Ward TR (2003) Expression and purification of a recombinant avidin with a lowered isoelectric point in Pichia pastoris. Protein Expr Purif 32 (2):167–174, http://dx.doi.org/10.1016/j.pep.2003.09.001

http://www.springer.com/978-3-662-43835-0

Commercial Plant-Produced Recombinant Protein Products
Case Studies
Howard, J.A.; Hood, E.E. (Eds.)
2014, XII, 281 p. 33 illus., 17 illus. in color., Hardcover
ISBN: 978-3-662-43835-0