Chapter 3 An Integrated Approach to Plant Bioproduct Production



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Chapter Highlights

- The area of plant bioproducts comprises several interdependent and allied disciplines, including plant breeding, agronomy, biotechnology, biorefining, the social sciences, and legal expertise.
- Whereas plant breeding involves random genetic changes in many genes, genetic engineering specifically alters or introduces only a very small number.
- Plant breeding and biotechnology benefit from the widespread use of "omics" approaches, including genomics, transcriptomics, proteomics, and phenomics.
- Creative solutions need to be developed for the efficient utilization of biomass using a biorefinery approach, where waste is minimized.
- Social scientists can help determine where bottlenecks may lay in terms of public acceptance, the relevance of research to society, and possible ethical considerations.

3.1 Introduction

Bioproducts can be produced from feedstock derived from plant biomass in various ways. These processes can be chemical, biological, or combinations of chemical and biological processes. Chemists can use **green chemistry** to convert substances, such as plant triacylglycerol, into various polymers including nylon and foams. A classic example of a biological process is the use of yeast to convert glucose into ethanol.

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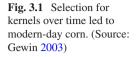
Enzymes, which can be isolated from living systems, can also be used to catalyze bioconversions such as the breakdown of cellulose to provide glucose units for ethanol formation. The term **biocatalysis** refers to the use of enzymes or cells to speed up chemical reactions. In addition, enzymes, in their own right, often represent useful bioproducts. As we will see in later chapters, various bioproducts can be derived from plant lipids, carbohydrates, and proteins using chemical and biochemical processes. In addition, many small molecules from plants can serve as building blocks or platform biochemicals to produce a plethora of fine chemicals that are often generated from petrochemical sources.

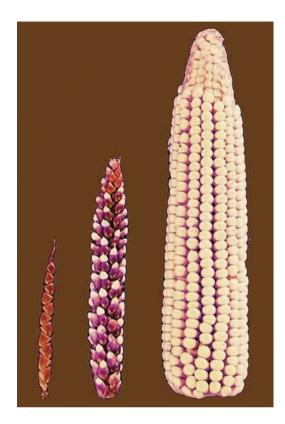
The area of plant bioproducts would not be fully realized without the interdependence of several allied disciplines. Within the last several decades, **plant breeding** and **agronomy** have had substantial impacts on global crop production. More recently, plant **biotechnology** has shown promise in crop improvement as a complement to modern plant breeding and in the engineering of microorganisms involved in **bioconversions**. In a **biorefinery**, processes are developed for maximum utilization of plant components. Finally, the social sciences and legal expertise are critical in dealing with issues such as consumer acceptance of bioproducts, intellectual property issues, freedom to operate, and dealing with trade barriers. This chapter provides insight into how bioproduct production can benefit from the integration of various disciplines.

3.2 Plant Breeding and Agronomy

In order to explain how plant breeding and agronomy have contributed to bioproducts, we will use corn (maize; *Zea mays*) as a notable example. Modern-day corn is very different from its wild ancestor (Fig. 3.1). It is estimated that the domestication of corn and selection for desirable traits began 6000–10,000 years ago (Gewin 2003). Today, corn is the most extensively grown field crop in the Americas. Corn is used as livestock feed, for human food (in raw or refined form), and as feedstock for producing various bioproducts. Corn can be separated into various fractions such as the endosperm, germ, and pericarp (the hull) (see Chap. 11 and Fig. 11.2 for details). The endosperm is enriched in starch and protein, whereas the embryo and pericarp are enriched in oil and **fiber**, respectively. These biochemical compounds have a variety of end uses which include the production of batteries, disposable diapers, and wallpaper (www.ontariocom.org/classroom/products.html).

Over time, the technique of crop improvement through plant breeding evolved into an elite science. Plant breeding involves the active selection of individuals with desirable traits. Plant breeding has been described in detail in textbooks such as Acquaah (2012). The discipline relies on an in-depth knowledge of genetics which is a branch of biology that deals with the heredity and variation of organisms (www. merriam-webster.com/dictionary/genetics). Methods used in plant breeding include basic selection for an individual trait, **hybridization**, **polyploidy**, and induced mutations. Plant breeding further involves controlled crosses between individuals





possessing desired traits. In cross-pollination, pollen from the anther of a flower from one plant is transferred to the stigma of a flower from another plant. In self-pollination, the pollen from the anther of a flower is transferred to the stigma of flowers on the same plant. Polyploid cells and organisms contain more than two paired sets of chromosomes (diploid) and often outperform their diploid relatives (Sattler et al. 2016). Hybridization involves the crossing of two highly inbred parental lines, each possessing one of the desired characteristics, to obtain progeny with both traits. Hybrids often exhibit increased hybrid vigor (often referred to as heterosis) which may include increased seed size, vigor, fertility, and overall productivity, involving gene-environment interactions. In contrast, the inbred parental lines used to produce the hybrids are often low-yielding. Natural mutations in the DNA of plants occur at low frequencies. Mutations, however, can be induced randomly through the use of chemical mutagens. Thus, plant breeders often use random chemical mutagenesis to develop sources of new traits.

Agronomy is defined as a branch of agriculture dealing with field crop production and soil management (https://www.merriam-webster.com/dictionary/agronomy). Breeding advances and various agronomic factors have contributed to a steady increase in corn yields over the past seven to eight decades. The application of commercial fertilizers to corn fields began in the mid-1940s with almost all of the **Table 3.1** Increases in USAcorn yield since 1866

Year(s)	Approximate yield (bushels/acre)
1866–1940	24.3–28.9
1970	72.4
2017	176.6

Source: US Department of Agriculture (https:// www.nass.usda.gov)

current acreage being fertilized. Herbicide application has also been critical for early season control of weeds so as to facilitate early planting of corn. Yield increases in USA corn since 1866 are depicted in Table 3.1. Today corn is grown in monoculture or alternated with soybeans (*Glycine max*).

3.3 Plant Biotechnology, Genomics, and Modern Plant Breeding

Biotechnology is the use of living organisms or biological processes for the purpose of developing useful agricultural, industrial, or medical products, especially through the use of methods such as genetic engineering (http://www.thefreedictionary.com/biotechnology). **Plant genetic engineering** involves modifying the DNA of a plant or introducing DNA from another source to generate a crop with a specific beneficial trait. The altered crops are referred to as being genetically engineered (GE) or genetically modified (GM). GE crop is a preferred term since plant breeding also involves genetic modification, especially chemical mutagenesis-assisted breeding where mutations are randomly introduced into the plant's DNA. Hildebrand (2008) provides a concise discussion of biotechnology and crop improvement. The reader is also encouraged to consult a special issue of the journal *Biocatalysis and Agricultural Biotechnology* which focuses on "trait introduction methods and innovation platforms in plant biotechnology" (Kovalchuk and Weselake 2014).

Genetic engineering is more precise than breeding with the generation of lines containing introduced traits occurring in a shorter time frame. Traditional breeding is a time-consuming process which requires generations of repeated and controlled selections and phenotyping. A **phenotype** is "the observable physical or biochemical characteristics of an organism, as determined by both genetic makeup and environmental influences" (http://www.thefreedictionary.com/Phenotyping). For example, a crop may have been genetically engineered with the intent of producing larger seeds, which is a phenotypic observation. On the other hand, **genotype** has to do with "the genetic makeup of the organism" (http://www.thefreedictionary.com/genotype).

In order to fully appreciate plant genetic engineering, it is worthwhile to have some insight into the flow of genetic information. A detailed discussion of the flow of genetic information can be found in various biochemistry and molecular biology textbooks (e.g., Buchanan et al. 2015; Moran et al. 2012). The nucleus of a plant cell contains

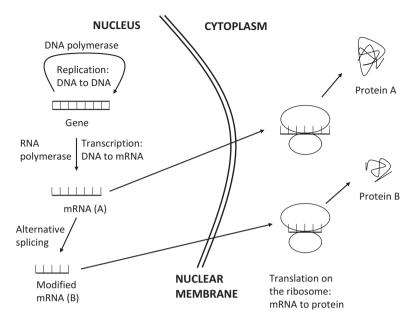


Fig. 3.2 The flow of genetic information in a eukaryotic cell and the involvement of alternative splicing. Replication, transcription, and alternative splicing occur in the nucleus, while translation (protein biosynthesis) occurs on the ribosomes in the cytoplasm. The image is based on information from Moran et al. (2012) and Syed et al. (2012)

chromosomes which in turn contain the "genetic blueprint" of the plant. This genetic blueprint contains genes which code for proteins and is reproduced during cellular replication. A **gene** can be defined as "the basic physical unit of heredity; it represents a linear sequence of nucleotides along a segment of DNA that provides coded instructions for the synthesis of mRNA, which, when translated into protein, leads to the expression of hereditary character" (http://www.dictionary.com/browse/gene).

More specifically, the DNA which makes up the genetic blueprint is replicated through the involvement of DNA polymerase, an enzyme driving the formation of a new double helix (Fig. 3.2). In turn, the conversion of DNA to mRNA is driven by RNA polymerase, which catalyzes the formation of a single strand of mRNA based on the information in the gene. In eukaryotic cells, mRNA, which also represents the information in the gene, moves outside of the nucleus to a protein formation factory where mRNA is converted to protein via a process known as translation or protein synthesis (Fig. 3.2). Individual genes are known to lead to the production of proteins and enzymes that can have biological functions in the plant. Initially, it was thought that one gene leads to the formation of one protein. We now know, however, that there are considerably more proteins than genes in a plant. Alternative splicing can lead to the formation of more than one protein based on the information from one gene (Fig. 3.2). In alternative splicing, "precursor mRNAs are spliced differentially to generate different mRNA isoforms" (Syed et al. 2012).

Unlike genetic engineering, in traditional plant breeding, thousands of genes are combined each time with the hope of changing traits. Often "backcrossing" is needed to compensate for unwanted side effects. In addition, plant breeding is only possible between closely related species. With genetic engineering, genes can be targeted and changed in specific ways. The process of producing mRNA from a gene can be enhanced and this is often referred to as overexpression. Through overexpression, the quantity of a specific enzyme in a plant cell can be increased. In contrast, through gene knockout, it is possible to eliminate the action of a specific enzyme in a plant cell. Genes can also be introduced from other sources through heterologous expression which involves the "expression of a gene or part of a gene in a host organism which does not naturally have this gene or gene fragment" (https://en.wikipedia.org/wiki/Heterologous expression). The general methods involved in genetic engineering are often referred to as recombinant DNA technology since they involve the manipulation of DNA. Interestingly, many of the techniques used in modern plant breeding are based on molecular biology and recombinant DNA technology (Moose and Mumm 2008). It is useful to think of plant genetic engineering as being complementary to plant breeding.

Agrobacterium-mediated transformation and microprojectile bombardment are two commonly used approaches for introducing genes from other sources into crop plants (Hildebrand 2008; Weselake 2011). In the case of Agrobacterium-mediated transformation, the gene of interest is inserted into Agrobacterium along with an antibiotic resistance marker and then used to infect plant cells. In turn, antibiotic selection is used to identify plant cells that carry the gene of interest. In microprojectile bombardment, particles coated with the gene of interest are propelled into plant tissue followed by regeneration of the altered or "transgenic" plants. Both methods of transformation are dependent on techniques in plant tissue culture, which can also be thought of as part of plant biotechnology. Transformed plant cells are grown in culture under conditions to promote shoot and root development, and the plantlets are eventually transferred to soil. In plant biology research, initial transformations are often conducted with the model plant Arabidopsis thaliana, a member of the Brassicaceae family which has a relatively short propagation time and small genome, with extensive genetic and phenotypic resources available (Koornneef and Meinke 2010). A genome is an "organism's complete set of DNA, including all of its genes" (https://ghr.nlm.nih.gov/primer/hgp/genome).

The first major crop traits, introduced through genetic engineering, were insect resistance and resistance to broad-spectrum herbicides, such as RoundupTM (glyphosate) (Hildebrand 2008). Both of these traits have been of great benefit to producers. *Bacillus thuringiensis* (*Bt*) is a soil bacterium that occurs naturally and makes a protein that is toxic to certain types of insects. The bacterium has been used a biopesticide since the 1920s. The gene coding for the toxic protein from *Bt* was introduced into corn resulting in a transgenic crop with a reduced need for application of commercial pesticides, which also results in reduced greenhouse gas emissions due to fewer passes of pesticide-dispensing airplanes. The herbicide resistance trait allows producers to apply a broad-spectrum herbicide to a crop so as to give it a competitive advantage against weeds. Glyphosate-resistant soybean is grown

extensively in the USA (Hildebrand 2008), while glyphosate-resistant canola (*Brassica napus*) is grown extensively in Canada (Weselake 2011). Unintended benefits of herbicide-resistant canola have included diminished fuel costs for farmers and reduced soil erosion because of increased use of zero-tillage practices where dead plant material remains unplowed in the field (Weselake 2011).

More recently, genetic engineering has contributed to the development of crops producing nutritionally enhanced and value-added compounds for industrial applications. The latter is in line with the focus of this book; i.e., genetic engineering can be used to modify plant metabolism so as to produce an enriched source of feed-stock for the production of bioproducts. The term **metabolic engineering** is often used to describe the redirection or modulation of carbon flow through a metabolic pathway (Stephanopoulos 2012; Venglat et al. 2014). It involves the adjustment and optimization of genetic and metabolic processes to produce a certain substance (https://en.wikipedia.org/wiki/Metabolic_engineering).

The development and commercialization of a GE crop is a very expensive undertaking which can only be effectively achieved by biotechnology companies with deep pockets. Extensive growth chamber, greenhouse, and confined field testing are conducted following the development of a GE line. GE corn, canola, soybean, and cotton (Gossypium hirsutum) account for 99% of the world's GE crops with the USA producing 40% of the global GE crop area (https://cban.ca/gmos/products/on-themarket/). Despite the resistance of the European Union (EU) to the adoption of GE crops, some insect-resistant GE corn is grown in Spain, Portugal, the Czech Republic, Slovakia, and Romania. GE crops grown in Canada include canola, corn, soybean, sugar beet (Beta vulgaris), and alfalfa (Medicago sativa; for animal feed), which accounts for about 6% of the global acreage of GE crops. In Canada, the Canadian Food Inspection Agency (CFIA) and Health Canada ensure that all GE products are safe for animals, people, and the environment. All plants with novel traits (PNTs) are regulated by the CFIA. A PNT is defined as "a plant that contains a trait which is both new to the Canadian environment and has the potential to affect the specific use and safety of the plant with respect to the environment and human health. These traits can be introduced using biotechnology, mutagenesis, or conventional breeding methods" (http://www.inspection.gc.ca/plants/plants-with-novel-traits/eng/1300137 887237/1300137939635). The introduction of a PNT as a commercial crop involves several stages of testing and approval. Assessing the environmental biosafety of PNTs involves evaluation of potential to become a weed or to be invasive of natural habitats, for gene flow to wild relatives, and to become a plant pest. The potential impacts on nontarget species and biodiversity are also assessed. The seeds of GE crops are considered to be the intellectual property of the company who holds the patent on the novel trait. Producers must purchase seed under license with the company and are not allowed to save seed for planting in the next year.

Consumer acceptance of GE crops is a controversial topic with the EU being very resistant to the widespread introduction of these crops (Sprink et al. 2016). One area of intense discussion is whether or not food products obtained from GE crops should be labeled as such. Environmental activist groups have also been concerned with the effect of insect-resistant GE crops on nontarget insects such as the monarch butterfly

(*Danaus plexippus*) (Hildebrand 2008). Insect-resistant corn, however, causes no immediate harm to monarch butterflies under field crop conditions. The view of environmental groups is interesting given that these groups also argue against using chemical-based pesticides which can potentially contaminate the environment. There has also been concern whether the herbicide resistance of GE crops can be transferred to non-GE crops or weedy relatives of these crops growing in the vicinity of GE crops. Contamination of a regular crop by a GE version of the same crop can occur via pollen flow and/or seed movement (Jhala et al. 2009; Weselake 2011). Maintenance of both isolation distances and isolation in time can be used to reduce gene flow from engineered oilseed species. Control of volunteer transgenic plants appearing in subsequent years can also reduce undesirable gene flow.

The flax (*Linum usitatissimum*) cultivar Triffid represents an interesting case of a GE crop (https://cban.ca/gmos/products/not-on-the-market/flax/). Triffid is a herbicide-resistant GE flax that was developed at the Crop Development Centre of the University of Saskatchewan. The name Triffid was based on John Wyndham's 1951 horror novel entitled *The Day of the Triffids* (Wyndham 1970).

Although Triffid was approved for release in Canada in the late 1990s, it was never grown on a large scale and was voluntarily de-registered in 2001 because of pressure from flax producers who wanted to protect their European markets. In 2009, however, traces of Triffid were found in Canadian flax shipments to Europe. This resulted in severe consequences for the Canadian flax industry. At the time Triffid contamination was found, the European market accounted for about 70% of Canada's flax exports.

Although there has been great concern regarding the manipulation of a single gene, edible products from crop plants modified through chemical mutagenesis appear to fly under the radar. Unlike GE crops, in most countries, chemical mutagenesis-induced variation applied to breeding is not regulated. One notable example is the development of flax with seed oil enriched in linoleic acid (18:2 $\Delta^{9cis,12cis}$) instead of α -linolenic acid (18:3 $\Delta^{9cis,12cis,15cis}$) (Green 1986; Rowland et al. 1995). Flax enriched in linoleic acid is broadly known as LinolaTM and as Solin in Canada (Hall et al. 2016). The decrease in unsaturation in high-linoleic acid flax oil resulted in oil more similar to corn oil and rendered it more suitable for cooking applications. Eventually, it was determined that this trait was the result of the inactivation of genes encoding fatty acid desaturase enzymes which govern the formation of α -linolenic acid from linoleic acid (Vrinten et al. 2005). Given that chemical mutagenesis was used to produce this type of flax, one would expect other mutations in the chemically treated genome. Random mutations introduced through chemical mutagenesis do not appear to be a concern with many of those who oppose the development of GE crops. Eventually, genetic engineering was used to further reduce unsaturation in high-linoleic flax so as to produce a GE line of flax with high oleic acid $(18:1\Delta^{9cis})$ content in the seed oil (Chen et al. 2015). Further information on the use of genetic engineering to produce oleaginous crops with modified lipid biosynthesis is presented in Chaps. 4 and 5.

Plant breeding has also benefited from **genomics**, which is "the study of the way genes and genetic information are organized within the genome, the methods for collecting and analyzing this information, and how this organization determines

their biological functionality" (Campos-De Quiroz 2002). "Omics" research works toward generating a global picture of genome DNA sequence and mRNA sequences leading to a plethora of proteins (Weselake 2011; Venglat et al. 2014; Gupta et al. 2017). The global analysis of mRNA sequences is known as **transcriptomics**, whereas the analysis of proteins is called **proteomics**. Omics now also includes **metabolomics**, which attempts to take a snapshot of the metabolite status of cell or tissue type. Very large amounts of genomic, transcriptomic, metabolomic, and proteomic data are analyzed by bioinformaticians or computational biologists. The reader is encouraged to consult Venglat et al. (2014) for a discussion on the genomics of seed development. A detailed knowledge of crop genomes has led to genomicsassisted breeding (Varshney et al. 2005) and the identification of new gene targets for genetic engineering (Weselake 2011; Venglat et al. 2014).

Plant phenomics is a relatively new "omics," which involves the high-throughput analysis of the phenome (Gro β kinsky et al. 2015; Tardieu et al. 2017). The crop phenome is represented by the structure and function of plants (i.e., all the phenotypic indicators). Gene variants and environmental changes can influence the plant phenome. Advances in plant phenomics are dependent on improvements in highthroughput noninvasive plant imaging and data analysis. In the future, investigations of plant phenomics could potentially lead to the generation of physiological predictors for complex traits, thereby linking genotype to phenotype for applications in plant breeding (Gro β kinsky et al. 2015).

Genome editing represents a relatively new development in plant biotechnology and allows biotechnologists to create site-specific changes in the plant genome (Kathiria and Eudes 2014; Yin et al. 2017). In contrast, *Agrobacterium*-mediated transformation and microprojectile bombardment are based on the random integration of DNA into the plant genome. So far, most applications of genome editing have involved loss-of-function through gene inactivation. It has been suggested that genome editing may be more acceptable to government regulators and consumers (Kathiria and Eudes 2014). The potential acceptability of genome editing as a method for introducing new traits into crops, however, is under intense discussion in the EU (Sprink et al. 2016).

Another emerging area in plant biotechnology is research aimed at developing crops that reproduce through asexual reproduction or **apomixis** (Barcaccia and Albertini 2013; Gewin 2003; Lovell et al. 2013). Through apomixis, fertilization is avoided altogether through the production of seed without pollination. Although apomixis occurs naturally in a few hundred species of plants, this biotechnology still needs to be effectively applied in a crop. In essence, seeds of apomixis crops could become natural clones of the "mother" with hybrid quality maintained from year to year by the producer.

Biotechnology is a very powerful tool for developing crops with desirable traits. Science-based evaluations support the safety of GE crops, but negative consumer perceptions still need to be overcome. Future applications of biotechnology for bioproducts will need to balance good science with consumer acceptance, while navigating an increasingly complex intellectual property landscape. Since bioproducts, in their strictest sense, are used only for industrial applications, this may be less concerning for consumers than the acceptance of edible products from GE crops.

3.4 The Biorefinery

Heating crude oil combined with fractional distillation can lead to several products which are differentially volatile based on temperature (Fig. 3.3). Petroleum distillates are used for liquid transportation fuels, lubricants, heating oil, and raw materials for the chemical synthesis of polymers and solvents. The mindset and engineering practices used in petroleum distillation can be applied to the processing of biomass. In biorefining, biomass is the raw material which can be separated into lipid, carbohydrate, protein, and high-value building-block biochemicals. In terms of plant biomass, many different feedstocks are possible, including crops and crop wastes, wood, sawdust, grasses, and algae. Biorefining also involves the conversion of the separate components into high-value bioproducts.

The biorefinery can be defined as "the sustainable processing of biomass into a spectrum of marketable products and energy" (de Jong and Jungmeier 2015). Creative solutions need to be developed for efficient utilization of biomass where waste is minimized. As an example, biodiesel production (see Chap. 4) uses seed oil, but the meal, which contains fiber and protein, is left over. Biodiesel production on its own is not fully profitable unless value can also be derived from the meal. Neibergs et al. (2016) have suggested that canola and *Camelina sativa* meal can be substituted for well-established soybean to supplement low-protein forages in livestock rations.

The Canadian Triticale Biorefinery Initiative (CTBI) research network was an example of a Canadian-based multi-coinvestigator research project aimed at wholeplant utilization (Beres et al. 2013a, b; King 2014). The CTBI was co-led by

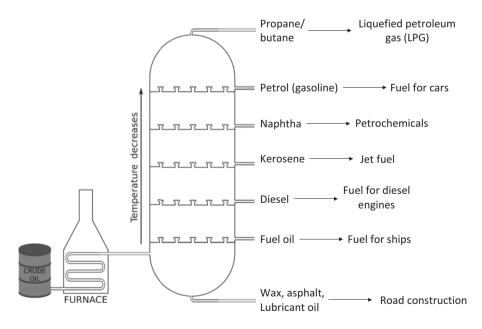


Fig. 3.3 Fractionation of crude oil (fossil fuel) by distillation to produce various products

Agriculture and Agri-Food Canada and Alberta Agriculture and Rural Development. Triticale (Triticosecale spp.) is a hybrid of wheat (Triticum aestivum) and rye (Secale cereale). The crop combines the high-yield potential of wheat with the disease and stress tolerance of rve. Lower input costs are required to produce triticale, and the crop does not naturally hybridize with other crops or wild species. Triticale has excellent potential for whole-plant utilization with applications in livestock feed, chemical production from green and mature biomass, bioethanol production from both the grain component and cellulosic material, polymer/fiber production, and pulp and paper production. Similar to corn (Fig. 11.2), the triticale grain has a relatively large starchy endosperm and small germ. Instead of a pericarp, the triticale grain has an outer bran layer. The grain contains about 65% starch, which can be used for bioethanol production, textile manufacturing, biodegradable packaging materials, and **adhesives**. Triticale straw, in turn, can be used for the production of cellulose, hemicellulose, and lignin. The cellulose can be used for bioethanol production, paper products, and cellulose-based composites. The hemicellulose and lignin fractions can be used to produce a range of specialty chemicals. Some of the genetic engineering objectives of the CTBI included modification of starch composition for specific applications, increasing seed oil content for livestock feed applications and reducing lignin content to increase the amount of cellulose harvested. The CTBI resulted in a valuable research platform which combined conventional and biotechnology-based breeding (King 2014). Chapter 11 of this book focuses on the biorefining of seeds of major crops to produce value-added substances.

Bioconversion (or biotransformation), which can be used in biorefining, is "the conversion of organic materials, such as plant or animal waste, into usable products or energy sources by biological processes or agents, such as certain microorganisms" (https://en.wikipedia.org/wiki/Bioconversion). A notable example is the bioconversion of starch or cellulose into bioethanol (see Chap. 6). Bioconversion can involve chemical, enzymatic, and/or microbial-facilitated processes. Biotechnologists are also interested in modifying metabolism in microorganisms such as bacteria and yeast. Indeed, applications of genetic and metabolic engineering were applied to microorganisms before plants (Vitorino and Bessa 2017). The burgeoning field of **synthetic biology** overlaps with metabolic engineering in that both disciplines are concerned with the modification of biochemical pathways in cells. Synthetic biology, however, is focused more on the use of synthetic DNA and genetic circuits to produce value-added products (Stephanopoulos 2012). Most applications of synthetic biology have been in microbial systems.

3.5 Bioproduct Development and the Social Sciences

Agricultural biotechnology has also drawn upon the expertise of social scientists and legal experts. Indeed, large-scale agricultural genomics projects funded by Genome Canada routinely include a GE³LS component where G = Genomics; E^3 = Ethical, Environmental, Economic; L = Legal; and S=Social Aspects (https://www.genomecanada.ca/en/programs/ge3ls-research). GE³LS research occurs at the crossroads of genomics and society.

Brewin and Malla (2012) have examined the effects of biotechnology on the Canadian canola industry. The success of canola as a major Canadian crop is due largely to public research, but more recently the private sector has been heavily involved in further improving the crop. Only a few major companies appear to exert influence over research on canola and the development of new varieties. Although the introduction of biotechnology and modern plant breeding led to a large increase in private investment into canola research, basic research and development on canola has been affected by intellectual property rights and freedom to operate issues. For example, the need for gene trait cross-licensing agreements has led to economic barriers for commercialization of new varieties.

Grierson et al. (2011) have compiled "one hundred important questions facing plant science research," many of which are relevant to plant bioproduct production. Some of the relevant questions include:

- "When and how can we can we simultaneously deliver increased yields and reduce the environmental impact of agriculture?
- How do we ensure that sound science informs policy decisions?
- Can we improve algae to better capture CO₂ and produce higher yields of oil or hydrogen for fuel?
- How can we use plants as the chemical factories of the future?"

The above questions and the many other questions compiled by Grierson et al. (2011) can be useful exercises for senior undergraduate students in plant science. The final chapter of this book by Smyth and Lubieniechi (Chap. 12) addresses the food versus fuel debate. In essence, can we effectively produce biofuels without affecting the food supply for a growing global population?

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