

Interdisciplinary, Cross-Supply Chain Approaches to Food Systems Improvement

A Dissertation

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Dedication

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Prologue

Guinea pigs have long served as important models for the nutrition field. At the start of my graduate career, I wanted to create a new kind of experiment, with an N=1 using myself as the guinea pig. I wanted to use my graduate experience as an opportunity to demonstrate interdisciplinary, cross-sector training and research to improve the relevance of nutrition by creating meaningful food systems change.

In the summer between my undergraduate and graduate studies, I reflected on the relevance of the nutrition field. I realized the field felt increasingly disconnected from the rest of the food system. Sure, everyone recognizes nutrition and health outcomes as critical food system factors, yet we fail to optimize this system to deliver high quality nutrition at scale. Often, we fail to adequately address nutrition until it is too late. We prescribe visits to dietitians after patients experience heart attacks. Then we expect patients to change the diet they've cultivated over a lifetime. This seems like an unsustainable model, particularly for a field that could perfectly align with preventative healthcare. As nutrition professionals, we often lament that the supply chain is built to deliver calorie-dense, nutrient-light foods at a low price. Yet, we must ask ourselves, have we effectively collaborated across disciplines, across sectors, and across the supply chain to develop more nutrition sensitive food systems? Given the last 30+ years of biomedical-based research and the dramatic increase in diet-related chronic disease over this timeframe, it seems systems approaches are more important than ever before. This principle forms the basis of this dissertation.

I wanted to demonstrate how one might pursue a systems approach by working across disciplines and across the supply chain. This was a difficult concept to articulate, especially when institutional processes often disincentivize cross-disciplinary collaborations. Faculty and graduate students are judged by our “impact on the discipline,” not by our abilities to create meaningful change throughout a system. This structure creates silos and rigid disciplinary boundaries that are difficult to transcend. A mix of curiosity, academic freedom, flexibility, perhaps an ounce of stubbornness, and a willingness to take risks pushed my mindset outside this boundary. Most of all, I believed in the work and became obsessed with creating a path to fully experience cross-disciplinary, cross-supply chain collaboration through my graduate work.

I was interested in developing food sources that contribute to economic vitality, health, social wellness, and environmental benefits. Growing up on a farm in rural South Dakota helped me see that each of these factors is critical in creating a sustainable system. Recently, big businesses throughout the agri-food industry have become demonized in the public eye. It is clear to me that creating sustainable solutions requires alignment between the industry, academia, non-profits, and the government sectors. I had a keen interest in collaborating with these groups throughout my graduate training. Within this broad framework, I was particularly interested in systems economics and the impact of upstream supply chain processes (e.g. plant genetics) on food quality. These crucial concepts are entirely missing from the current nutrition curriculum. Further, I hoped that my graduate work would provide a wide range of experiences, skills, and abilities across food systems disciplines and sectors, as I fully believed that these skills and abilities

would increase my effectiveness as a food system professional. Luckily, and with considerable effort, we found the right collaborators and the right project, at the right time. The rest of this dissertation describes the projects we pursued in laying out an initial framework and demonstrating the value of interdisciplinary, systems-based research and training nested in a traditional graduate program. This includes a literature review on food systems approaches, a whole grain systems analysis, a genetics study aimed at improving pennycress seed meal quality, an economics study estimating pennycress production economics, a review of the role of the land grant university in developing sustainable food systems, and a paper discussing our approach to interdisciplinary food systems research and training.

Chapter 1: Introduction – framing, context, and mindset

Abstract

The field of nutrition is rapidly evolving into a new paradigm characterized by the complex, adaptive, wicked challenges faced by food systems professionals. Systems approaches are required to manage the complex issues at the intersection of the food system, the environment, and human health. Despite these complex, interlinked challenges, nutrition research and training remain siloed. Given the current landscape of systems problems, new systems-based approaches to research and training are required. Similar to the nutrition ecology framework, these approaches are requisite to the successful management of the health, environmental, economic and societal implications based on complex food system actions. In that vein, this work provides an initial framework, along with examples of hands-on experiential learning opportunities within a nutrition graduate program focused on systems approaches to nutrition. This is demonstrated through interdisciplinary collaborations across the supply chain and food system. First, we seek to understand supply chain barriers to whole grain availability and access in restaurants. Then, we shift focus to the development of a new sustainable crop, pennycress. Here, we collaborate upstream in the supply chain with plant geneticists to identify genetic targets to improve the quality of raw materials. Then we collaborate with economists to model production economics. Overall, this approach mixes adapted socio-ecological, biological, and economic analyses to provide a more holistic perspective to food systems development. Then we discuss the role of the land grant university in developing sustainable food systems, and we discuss learnings from our interdisciplinary, systems training approach. Finally, based on the work as a whole, we provide recommendations regarding a three-step process to catalyze future systems approaches in nutrition.

1.1 Introduction

This dissertation is different from what one might consider classical or standard graduate nutrition (PhD) work. This is both by nature and by design. Clearly, each graduate experience is unique, yet this work explicitly reached outside traditional disciplinary boundaries to create a unique package meant to equip the author with skills required to address 21st century, wicked, food systems and nutrition challenges.

The field of nutrition is rapidly changing. Nutrition professionals can no longer rely on past, reductionist approaches to address pressing societal challenges. The current environment necessitates that we adapt to develop and implement multiple-disciplinary systemic approaches. Recognizing this challenge, the author, graduate advisor, and graduate committee developed a course of study that integrated biological science, economic analyses, and qualitative research to critically understand the challenges, barriers, and opportunities associated with developing sustainable, nutrition sensitive food systems. Equally important, the dissertation offers a critical evaluation of the philosophical and theoretical issues associated with the nutrition discipline. The chapters of this dissertation describe and discuss these components, oftentimes integrating each component.

The following sections are organized as follows:

Chapter 2: Literature Review: The Case for Nutrition-Systems Approaches

Chapter 2 discusses a range of topics critical to the current state of the nutrition discipline. Here, the last 150 years of nutrition research are summarized. This summary highlights the successes of the reductionist approach to nutrition, yet it argues that we have clearly entered a new paradigm requiring systems approaches that integrate several sectors and disciplines to manage grand nutrition challenges. This is described as a nutrition-systems approach. This leads into a discussion of wicked problems and describes the diet-health-environment trilemma as a clear example of a wicked challenge facing the field. Sustainable diets and sustainable dietary recommendations are briefly described to provide further context regarding a shift in mindset. Finally, examples of systems approaches are presented to give further global context of a shift toward new methodologies. The nutrition sensitive value chain approach is briefly described as an example being implemented across the globe.

In the spirit of thinking globally and acting locally, the Minnesota agriculture and food landscape are briefly described. This includes a discussion of key food production and food security issues. This chapter further discusses healthful, potentially sustainable seed-based foods that might fit into the Minnesota food environment, including whole grains and new cash cover crops. Whole grain health considerations are briefly described, followed by a more thorough discussion of a new crop called field pennycress.

Integrating whole grains and sustainable crops, like field pennycress, into the diet has the potential to improve both planetary and human health, all while aligning with current consumer and business trends. However, key supply chain barriers must be overcome. The rest of the dissertation describes and discusses efforts to overcome these barriers.

The barriers associated with pennycress development are thoroughly described as further chapters aim to address these issues.

Chapter 3: Challenges and opportunities associated with whole grains use in Twin Cities restaurants: a food systems perspective

Chapter 3 describes a study conducted to understand the key challenges and barriers associated with using whole grains in Twin Cities restaurants. The paper employs a qualitative, on-the-ground interview-based approach to understand the whole grain supply chain and the macro issues surrounding whole grain production and consumption. The chapter shows that a lack of knowledge around whole grains, lack of production incentive, and lack of consumer demand results in decreased whole grain use in restaurants. Perhaps more importantly, this chapter demonstrates a system-wide approach to understanding key challenges and barriers associated with developing and delivering healthful foods.

Chapter 4: Identification of reduced glucosinolate mutants in field pennycress (*Thlaspi arvense L.*) by large-scale screening of mutant populations through forward and reverse genetics

Chapter 4 describes a study designed to identify mutant pennycress lines with low glucosinolate profiles and to identify potential causative mutations. Similar to other cash oilseed crops, reducing anti-nutritive components in seed meal improves seed meal utility as a food and feed source. The paper employs a forward and reverse genetics approach to understand potential candidate mutations that can be combined with other beneficial traits

to develop a food-grade pennycress line. The chapter describes two mutations with significant effects on pennycress glucosinolate production. One mutation occurs in the core structure biosynthesis gene CYP83A1, which results in a ~19% reduction in glucosinolate accumulation. Similar effects are observed in Arabidopsis plants harboring similar mutations. Another mutation occurs in the transcription factor MYC3, which results in a ~45% reduction in glucosinolate accumulation. Glucosinolate production decreases in the model plant Arabidopsis when two other transcription factors, MYC2 and MYC4, are also mutated. These plants also experience decreased plant fitness. Our observation that pennycress plants harboring a mutation in the single transcription factor MYC3 exhibit decreased glucosinolates without negative impacts on plant fitness implicates MYC3 as a potential novel regulator in pennycress glucosinolate production. These mutations can potentially be combined with other beneficial mutations to produce a pennycress line suitable for food use.

Chapter 5: Understanding on-farm production economics of canola reveals potential economic impact of field pennycress

Chapter 5 describes an economic modeling study conducted to establish a potential base line for food grade pennycress production. On-farm production and economic data were collected for canola from farms in North Dakota and Minnesota, as these are two of the largest canola producing states in the Upper Midwest. Factors critical to revenue (e.g. commodity pricing, yield) and expenses (input costs, labor, management costs) are averaged on a per-acre basis over ten years. Prices, expenses, and management practices were assumed to be similar for pennycress production. Revenue and expenses are

compiled into a per-acre producer enterprise budget. Using these data, we determine breakeven yield (i.e. where farmer returns equal expenses at a given price point) for the pennycress system. Different yield scenarios are generated to determine the impact of pennycress yield on return. Yield scenarios are generated based on values reported in the literature. The paper then estimates the potential economic impact of the three-crop (corn-pennycress-soybean) system. On-farm production and economic data are collected from the same region and averaged over a ten-year period. Potential soybean yield penalties of crops planted following pennycress are estimated based on values reported in the literature. This chapter finds that adequate pennycress yield is essential for the economic output of the system. Yield must reach 1495 lb/acre to break even, and return will vary significantly depending on yield variation. The return of the corn-pennycress-soybean rotation will vary significantly depending on soybean yield reductions. Taken together, data indicate that breeders should aim to reach a minimum yield of 1495 lb/acre and ensure that pennycress can be harvested to allow for adequate time for soybean production.

Chapter 6: Intersection of diet, health, and environment: land grant universities' role in creating platforms for sustainable food systems

Chapter 6 briefly reviews the influence of diets on health and environmental outcomes in the context of global sustainable dietary recommendations. The paper discusses the role of land grant universities in developing nutrition sensitive, environmentally resilient food systems. The chapter describes the need for more systems approaches throughout academia and identifies potential barriers to implementing these approaches. Mechanisms

to facilitate these approaches, such as community-based participatory action research and the restructuring of institutional processes, are identified. Then the article discusses local food system initiatives in Minnesota and offers insight into how the University of Minnesota may play a role in developing nutrition sensitive value chains by coordinating and supporting local food efforts. Ultimately, this chapter suggests that land grant universities can help local communities equitably collaborate with upstream and downstream value chain actors and external influencers to develop nutrition sensitive, environmentally resilient value chains.

Chapter 7: Critical reflections on a multi-disciplinary training framework for the fields of agriculture, food, and nutrition sciences

Chapter 7 critically reflects on the multiple disciplinary training and research approaches that contributed to this dissertation. The paper discusses critical learning and training experiences that support the concept of the “T-shaped professional.” Multi-sector, multiple disciplinary experiences occurring internal and external to the university are briefly described followed by a discussion on how these experiences fit within the practical context of the 21st century agriculture, food, and nutrition professional. This chapter argues that more training experiences and graduate programs should follow a similar approach and provides examples occurring in other disciplines and sectors. The paper provides insight into how to facilitate multiple disciplinary collaborations and discusses limitations and strengths of this type of approach.

Chapter 8: Conclusions – Toward the development of nutrition systems approaches

Chapter 8 summarizes conclusions of each chapter and positions them in the context of nutrition systems approaches. The paper provides a framework for other researchers and professionals to pursue a nutrition systems approach and outlines potential future research and training opportunities related to sustainable food systems, cash cover crop development, and whole grain consumption.

Chapter 2: A new era of nutrition research

2.1 Introduction

The field of nutrition has undergone a series of paradigm shifts over the past 200 years to address food and health-related challenges. Ridgway and colleagues summarize this evolution of the field under three general eras of nutrition science: the foundational era, the nutrient deficiency era, and the dietary excess and imbalances era (Ridgway et al., 2019). The foundational era (1700s-1930s) was characterized by the identification of basic chemical composition of foods along with the initial discovery of the micronutrients. Beginning in the early 1900s, nutrition science focused on reducing micronutrient deficiency-related diseases. Nutrition scientists successfully addressed major public health diseases, including niacin deficiency, pellagra, and the iodine deficiency, goiter (Leung et al., 2012; WHO, 2000). The 1940s ushered in the dietary excess and imbalances era. Nutrition science focused on the relationship between single foods, nutrients, and chronic diseases. Scrinis characterizes this period as the “good and bad” food era (Scrinis, 2015b). Nutrients and foods, such as fat, cholesterol, and eggs were stigmatized based on their perceived negative contributions to health. Dietary guidance, such as the Dietary Guidelines for Americans (DGAs) and the Reference Daily Intake (RDIs) emerged during this era, and it largely reflected the good and bad food paradigm. Starting in the 1990s, nutrition shifted yet again, into an era that Scrinis calls “functional nutritionism” (Scrinis, 2015a). In this period, nutrition became a marketing tool used to sell products based on functional characteristics and other positive messaging, (front of pack labeling: “superfood,” “high in antioxidants,” “organic,” “natural,” etc.).

According to Scrinis, reductionism largely served as the historical foundation of nutrition science, as expressed through scientific theory and practices within each of these general eras (Scrinis, 2015c). Reductionism refers to the process of dissecting and studying the smallest parts of a system rather than assessing the complicated interactions within and among components. Using a reductionist lens, the field has traditionally focused on single nutrients, single foods, and singular health outcomes. However, the discipline is evolving once more, away from reductionism, and toward holistic approaches focusing on the complex systems underlying food production and consumption (Fardet & Rock, 2018). Ridgeway and colleagues describe the current era as the food systems sustainability era (Ridgeway et al., 2019).

The food systems sustainability era of nutrition science places increased emphasis on the relationship between food production, consumption, health, and environmental outcomes. Some characterize this new paradigm as the “New Nutrition Science,” which focuses on holistic, systems-based approaches rather than adhering only to traditional biomedical science approaches (Cannon & Leitzmann, 2005). Inarguably, the approaches fostered during eras of reductionist nutrition science were effective at addressing nutrition problems with one-to-one cause-and-effect relationships, such as hunger and micronutrient deficiencies. However, these approaches are largely ineffective at addressing current nutrition issues, as exemplified by the last 30 years of biomedically-based research and the alarming increase in obesity (Roberto et al., 2015). These issues lack one-to-one cause and effect relationships and are influenced by many factors such as the eating environment, community, socioeconomic status, biological environment, food

supply chains, food quality, and nutritional genomics. To add further complexity, the food production system that contributes to chronic disease also contributes to environmental degradation. This relationship requires nutrition and food scientists to grasp at least a cursory understanding of the environmental impact of food production. Current nutrition issues necessitate that the field gravitates into a new era of systemic research approaches, accounting for social, economic, environmental, and health aspects of foods (Lee et al., 2017). Designing and executing these approaches first requires an understanding of the food system.

2.2 Food Systems and Wicked Problems

Developing new, systems-based approaches requires a thorough understanding of the food system and the wicked problems inherent to this integrated system. Food systems are complex, adaptive systems comprised of all actors, activities, drivers, influencers, and outcomes involved in the production, delivery, and consumption of food (Drewnowski et al., 2020). The 2017 High Level Panel of Experts on Food Security and Nutrition (HLPE), a research-policy interface mechanism of the United Nations, defined food systems in terms of the following components: food supply chains, food environments, consumer behaviors, and diets (HLPE, 2017). In this model, supply chains consist of on-farm production systems, storage and distribution, processing and packaging, retail, and markets. The food environment includes food availability and physical access; economic access; promotion, advertising, and information; and food quality and safety. Consumer behaviors include individual food choices, preparation methods, and other eating habits. Here, diets are defined by quantity, quality, diversity, and safety. These factors influence nutrition and health outcomes in addition to other social, economic, and environmental

impacts. Several other external drivers influence these components, and they include: biophysical and environmental; innovation technology and infrastructure; political and economic; socio-cultural; and demographic (HLPE, 2017). Biophysical and environmental drivers include natural resources, ecosystem services, and climate change. Innovation, technology, and infrastructure drivers include cutting edge technology enabling development and delivery of foods. Political and economic drivers include globalization, national and international leadership, humanitarian crises, food prices, and market volatility. Socio-cultural drivers include cultures, religions, rituals, social traditions, and empowerment of minority communities. Demographic drivers include population growth, changing age distribution, urbanization, migration, and displacement. Each of these drivers interact with food systems actors to influence political and institutional processes. The complexity of the food system creates a rife environment for wicked problems.

2.3 Wicked problems

Clearly, the food system is a complex, adaptive system comprised of multiple sectors, stakeholders, drivers, influencers, processes, and actors. Each of these components encompass several subcomponents, which cut across professional disciplines. Individuals and communities within these components have unique worldviews, mindsets, intensions, and cultures, further contributing to the complexity of the system. Given its complexity and its place at the nexus of human health and the biological environment, the food system produces wicked problems. The term “wicked problem” derives from the social planning literature and refers to challenges that are complex and interlinked; involve many stakeholders, sectors, and disciplines with potentially differing worldviews; and

lack clear cut, definitive answers (Rittel & Webber, 1973). Wicked problems contrast with “tame” problems, which have definitive solutions. While tame problems may be solved by a single discipline or sector, wicked problems require multi-disciplinary, multi-sector systems approaches (Dentoni et al., 2012). Wicked problems are often discussed in the context of agri-food systems. For instance, the relationship between food production, diet, health, and climate change is a clear example of a wicked food systems problem.

2.4 Wicked problems and the food system: the diet-health-environment trilemma

The global food system contributes to a litany of complex issues related to consumption and production. Currently, the global population faces the triple burden of malnutrition: chronic hunger, deficiencies in key micronutrients, and obesity (Gomez & Barrett, 2013). More than 820 million people experience hunger (FAO, 2014), 2 billion lack sufficient nutrients, and nearly 2 billion experience overweight and obesity (J. Fanzo et al., 2018). Many countries experience each of these types of malnutrition, particularly as they become more urbanized. As economic output increases diets shift from undernutrition to overnutrition, a phenomenon known as the nutrition transition (*The Nutrition Transition*, 2020). In the United States alone, obesity prevalence soared from 30.5% in 1999-2000 to 42.4% in 2017-2018, with severe obesity increasing from 4.7% to 9.2% during this timeframe (Center for Disease Control and Prevention, 2020). Other diet related diseases, such as heart disease, stroke, type 2 diabetes mellitus (T2DM), and certain cancers are leading causes of death worldwide (Center for Disease Control and Prevention, 2020). The National Center for Chronic Disease Prevention and Health Promotion reports nearly 30.3 million Americans have diabetes with T2DM accounting for 90-95% of these cases (CDC, 2017). Another 84 million Americans are prediabetic, which often leads to T2DM

if left untreated (CDC, 2017). More than 90 million American adults suffer from cardiovascular disease, and it is the leading cause of death, accounting for a third of all deaths in the United States (Benjamin et al., 2017). Prevalence of childhood and adolescent obesity has tripled since the 1970s, with recent data reporting that nearly 1 in 5 children aged 6-19 experienced obesity between 2015-2016 (Hales, 2017). Childhood obesity is associated with increased risk for adult morbidity and chronic disease such as T2DM, hypertension, metabolic syndrome, and cardiovascular disease (Biro & Wien, 2010; Rush & Yan, 2017). These ailments come with harsh economic implications. In the United States, the annual medical cost of obesity is nearly \$150 billion while heart disease costs \$219 billion (CDC, 2020) (Center for Disease Control and Prevention, 2020). Clearly diets play a key role in health and have tangible economic implications.

Meanwhile, agricultural food production systems, food choice, and dietary patterns are critical factors for environmental outcomes, like greenhouse gas emissions, decreased biodiversity, and water use (M. Clark & Tilman, 2017; Forouzanfar et al., 2015; Hallström et al., 2015). Over 40% of terrestrial land is dedicated to agricultural production (FAO, 2018). The Intergovernmental Panel on Climate Change estimates that global agriculture accounts for nearly 24% of all greenhouse gas emissions (Pachauri et al., 2015). Poor field management, such as improper fertilizer use, contributes to water pollution through runoff of excess nitrogen and phosphorus. This can lead to eutrophication of natural water bodies, contributing to fish kills and loss of aquatic life (The United States Environmental Protection Agency [EPA], 2020). The relationship between food production, consumption, and environmental outcomes is referred to as the

diet-health-environment trilemma (M. Clark et al., 2018). Negative health and environmental impacts from the food system may increase as the population exceeds 9 billion by 2050, and food production increases to keep pace with demand. Thus, food, nutrition, and agriculture professionals must feed a growing population diets that are both healthful and environmentally sustainable, which is an inherently wicked challenge.

The Food and Agriculture Organization of the United Nations conceived the Sustainable Development Goals (SDGs), which provide orientation for food and agricultural professionals striving to curb the diet-health-environment trilemma (FAO, 2017a). The SDGs include 17 cross-cutting development goals, all of which intersect with the sustainable development of nutrition-sensitive food systems (Barbier & Burgess, 2017). For example, SDG 3: Good Health and Wellbeing, SDG 9: Industry Innovation and Infrastructure, SDG 11: Sustainable Cities and Communities, SDG 12: Responsible Consumption and Production, and SDG 13: Climate Action all fall within the purview of food and agriculture professionals.

As a field, how do we capture the complexity and nuance of creating sustainable, nutritious food solutions that preserve the integrity of population and planetary health? The following sections review potential approaches for addressing this wicked challenge, including sustainable dietary recommendations and the development of nutrition sensitive value chains. First, we will review the nutrition ecology framework to provide orientation for a holistic approach to solving food systems challenges.

2.5 Nutrition ecology provides a framework to capture food systems complexity in the era of food systems sustainability

The nutrition ecology framework helps capture the complexity and interlinkages of food systems actions and outcomes. In contrast to previous reductionist nutrition paradigms, nutrition ecology integrates dimensions of health, environment, society, and economics (Schneider & Hoffmann, 2011). These dimensions are illustrated in Figure 2.1. In this context, the health dimension includes elements of food and nutrition security, like food quality, food safety, physiological health, and mental wellbeing. The environment dimension encompasses climate impact, use of natural resources, and other environmental considerations. The economic dimension encompasses markets, industries, and allocation of resources. The societal dimension encompasses sociocultural practices, politics, socioeconomics, ethics. The nutrition ecology framework considers each dimension simultaneously and co-equally throughout the food system, from production to consumption and all other influences (Schneider & Hoffmann, 2011). This is an inherently interdisciplinary concept, which integrates knowledge from different disciplines and practical fields on the conceptual and methodological levels (Schneider & Hoffmann, 2011).

The nutrition, environment, economic, and society dimensions of the nutrition ecology framework align with other experts and global leaders. The Food and Agriculture Organization broadly defines sustainable diets as those that maintain biodiversity, remain culturally appropriate and acceptable, optimize human and natural resources, produce low environmental impacts, and contribute to food and nutrition security for present and future generations (Burlingame, 2012). The nutrition ecology dimensions encompass the

complexity of this definition. Further, agricultural innovation that aligns with each of these dimensions is critical to develop sustainable food systems that achieve the Sustainable Development Goals (Barbier & Burgess, 2017; Drewnowski et al., 2020). Similarly, the HLPE advocates for cross-disciplinary, whole-systems collaborations to develop more sustainable, health food solutions, and these cross-disciplinary collaborations span the dimensions of the nutrition ecology framework (HLPE, 2017). Raiten and Combs, from the National Institutes of Health and the United States Department of Agriculture respectively, suggest that nutrition ecology provides an ontological approach to describe the complex relationships within the food system (Raiten & Combs, 2019). They suggest this approach is essential to develop food solutions that maintain individual biological systems, natural environment, and socioeconomic systems as specified by the HLPE and SDGs (Barbier & Burgess, 2017; HLPE, 2017). Altogether, the nutrition ecology framework provides a mechanism and epistemological foundation to address complex, wicked food systems challenges in the food systems sustainability paradigm (Ridgway et al., 2019). Using this lens, we discuss proposed sustainable dietary recommendations, nutrition sensitive value chains, local food security, and development opportunities for conventional (whole grain) and emerging (pennycress) food systems.

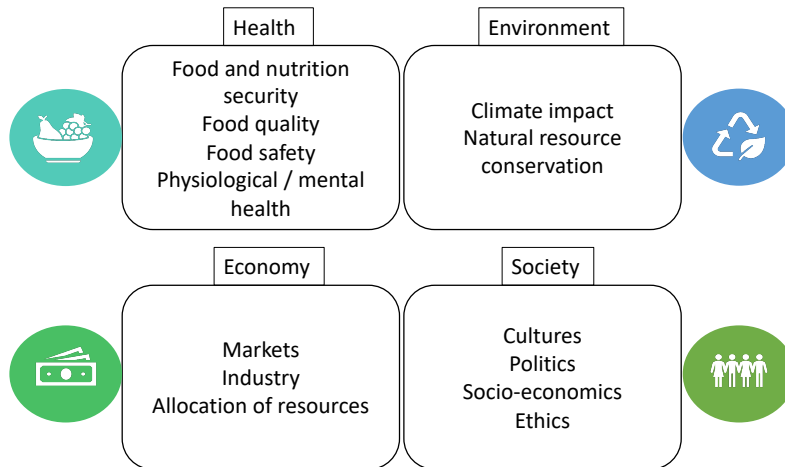


Figure 2.1 - Dimensions of the nutrition ecology framework capture the complexity of food systems implications

2.6 Sustainable dietary recommendations

Given the relationship between food production, consumption, health, and environmental degradation, sustainable diets have become increasingly relevant. Some suggest that incorporating sustainability elements into dietary recommendations is an essential approach to preserving population and planetary health (Willett et al., 2019).

There are four elements of sustainable diets, and they align with the nutrition ecology framework. Sustainable diets promote health and nutrition, minimize environmental impacts, remain economically feasible, and promote sociocultural wellness (Drewnowski, 2018). Healthful diets include adequate amounts of protein-rich foods, fruits, vegetables, and fats. Dietary recommendations vary depending on demographic characteristics. However, dietary guidance, such as the Dietary Guidelines for Americans (DGAs), is based on mean population requirements (US Department of Agriculture and US Department of Health and Human Services, 2015). The DGA-recommended dietary

pattern should support the health of the general population, but achieving dietary guidance is difficult as the food supply is largely non-compliant with DGAs (Krebs-Smith et al., 2010; P. E. Miller et al., 2015). Dietary guidance has not traditionally accounted for the impact of diets on the environment, which presents challenges and opportunities as we approach 2050.

A growing body of literature highlights the link between plant-based diets, nutrition, and environmental health. The EAT-Lancet report suggests diets promoting nutrition and physical health as well as environmental sustainability are essential for the persistence of the global population (Willett et al., 2019). Plant-based diets are associated with decreased mortality and decreased risk for chronic disease (Orlich et al., 2013; Satija et al., 2016, 2017). Increased vegetable and fruit consumption is associated with decreased risk for chronic diseases (Hung et al., 2004), including Type 2 Diabetes mellitus (T2DM) (Muraki et al., 2013), cardiovascular disease (CVD), cancer (Dagfinn Aune et al., 2017), and weight gain (Bertoia et al., 2015). Diets rich in whole grains are associated with decreased risk for T2DM, CVD, and cancer (Dagfinn Aune et al., 2016a; Parker et al., 2013). Similarly, nut and legume consumption is associated with decreased risks of CVD, T2DM, and all-cause mortality (Afshin et al., 2014; Dagfinn Aune et al., 2016b; Bernstein et al., 2010). This contrasts with diets high in animal-based foods, particularly red processed meats, which are associated with increased risk for chronic disease (G.-C. Chen et al., 2013; Feskens et al., 2013; Pan et al., 2012). Other studies indicate that plant-based diets have a reduced carbon footprint compared to diets high in animal-derived foods. A 2017 analysis showed that consumption and production of fruits, vegetables,

and grains have lower impacts on global warming via decreased greenhouse gas emissions compared to red meat (Clune et al., 2017). This supports a 2017 study assessing environmental impacts of agricultural production systems and food choice, which showed that animal-derived foods have a negative environmental impact compared to plant-based foods (M. Clark & Tilman, 2017). Other reports show clear links between dietary patterns and environmental impact, with plant-based diets having considerably reduced impact compared to those that include meat (Aleksandrowicz et al., 2016; Hallström et al., 2015; Nelson et al., 2016).

The parallel benefits of plant-based diets for human and environmental health may influence future dietary recommendations, as shown by the EAT-Lancet report (Willett et al., 2019). The report recommends a diet low in animal-derived foods like red meat and dairy and encourages increased vegetable, fruit, legume, and whole grain consumption (Willett et al., 2019). This is similar to the DASH and Mediterranean diets, which purportedly have lower environmental impacts compared to Western diets (Nelson et al., 2016). While this diet would likely improve human health and reduce greenhouse gas emissions, some question its one-size-fits-all nature. The WHO withdrew support due to questions around the feasibility and cultural suitability of the diet (*WHO Withdraws Endorsement of EAT-Lancet Diet*, 2019). The cultural significance of food and agriculture is largely absent from the report. Future iterations might benefit by incorporating inclusive, socio-cultural perspectives. Despite these limitations, EAT-Lancet provides evidence-driven insight into the influence of sustainable diets and sets goals for food systems professionals.

As dietary recommendations shift to include sustainability components consistent with the current nutritional paradigm, food systems activities must evolve to become more sustainable to keep pace with potential demand. In particular, supply chains must shift to adopt more sustainable practices and emphasize health throughout supply chain processes. Delivering sustainable, healthful foods might be achieved through a nutrition sensitive value chain approach.

2.7 Nutrition sensitive value chains: the value chain impact of nutrition

Developing foods and diets that sustain planetary and human health requires multiple disciplinary, systems approaches that account for nutrition, food science, agriculture, ecology, economics, policy, and socio-cultural factors (Barbier & Burgess, 2017; Tu et al., 2019). Systems approaches are becoming critical in addressing complex societal issues like obesity (T. T. Huang et al., 2009; Lee et al., 2017) and conservation agriculture policy (Ribeiro et al., 2016). However, these approaches are often difficult to conceive and coordinate due to diverse stakeholder interests, grand scale of operation, and broad scope of roles, functions, and activities. Nutrition sensitive value chains provide a framework for this type of approach.

The nutrition sensitive value chain approach contrasts with traditional nutrition approaches. Historically, malnutrition has been targeted through strategies aimed at controlling risk factors in the general population (Boyle & Holben, 2010). These strategies include disseminating information and recommendations intended to improve nutritional knowledge while enabling behavior change. While the classical approach to

community nutrition has leveraged promotional material (myPyramid, myPlate) and consumer-focused dietary guidance (e.g. Dietary Guidelines for Americans), citizens remain burdened with nutrient deficiencies and diet-related chronic diseases in both developing and developed countries (Troesch et al., 2012). In contrast, rather than simply rely on education and recommendations, the nutrition sensitive value chain approach integrates actors involved in the supply chain to create comprehensive solutions.

Food supply chains determine critical factors that influence diet and food choice, including price, availability, convenience, and safety. Supply chains include all actors and processes from production to consumption. As raw materials flow through the chain, key steps add value to the product, which gives rise to the term “value chain” (**Figure 2.2**). While the traditional value chain concept focuses solely on economic value, there is burgeoning interest in the concept of nutrition sensitive value chains (FAO, 2017a, 2017b; *Identifying Opportunities for Nutrition-Sensitive Value-Chain Interventions* | IFPRI, n.d.; Uccello et al., 2017). These aim to enhance supply and demand for nutritious foods while sustainably adding nutritional value at critical chain stages (FAO, 2017b). This framework examines links between nutrition issues in target populations and constraints in supply and demand for foods that may address these problems (de la Peña & Garrett, 2018). Value chain activities influence nutrition content, quality, and consumer acceptance. For instance, plant genetics influence the nutrient content of raw materials and ingredients, processing influences bioavailability, nutrient content and quality, and policies influence availability.

2.7.1 Plant Genetics and Nutrition

Upstream in the supply chain, plant breeding influences nutritional content and quality of foods. Biofortification leverages plant genetics to increase micronutrient content in staple foods such as corn, wheat, potatoes, or rice (Nestel et al., 2006). These foods are targeted given their dietary predominance in developing countries stricken with micronutrient deficiencies, such as anemia. Furthermore, while these foods tend to be rich in calories, they lack in micronutrient content. Theoretically, increasing micronutrient content of these staples would have the largest impact at mitigating micronutrient deficiencies.

Biofortification can be achieved through conventional breeding practices or through modern gene editing/bioengineering approaches. In a 2008 report, Harjes et al used a conventional breeding approach to increase pro-vitamin A content in maize. The researchers discovered significant natural variation in beta carotene content among a maize population which they exploited to produce a maize population with beta carotene levels of 13.6 ug/g compared to 0.5-1.5 ug/g in most maize varieties worldwide (Harjes et al., 2008). In total, the researchers identified four natural mutations in the beta carotene biosynthetic pathway that accounted for 58% of the variation. Using conventional plant breeding practices, researchers can introgress the mutated traits responsible for increased B-carotene into several maize varieties, which could help mitigate the burden of vitamin A deficiency in countries where maize is the staple crop.

Modern gene editing and bioengineering techniques can also be harnessed for biofortification. Notably, De Lepeleire and colleagues demonstrated folate biofortification in potato tubers (Lepeleire et al., 2018). Using a transgenic approach,

researchers over-expressed folate biosynthetic genes in potato tubers, which increased potato folate content twelve-fold from 165 ug/100 g dry weight in the wild type to 1925 ug/100 g dry weight in the modified plant. Researchers discovered that they could alter folate levels without sacrificing yield or water content. Additionally, the group found that folate levels remained elevated after nine months of storage, indicating that folate derived through this approach maintains stability for a significant period of time post-harvest.

Success of these crops hinge on consumer acceptability. In a 2017 study, Perez and colleagues examined consumer acceptance of iron-biofortified beans in South America (Pérez et al., 2017). The study aimed to identify socio-demographic characteristics that might predict consumer preference for key organoleptic qualities of the biofortified beans, including size, color, flavor, texture, and cooking time (Pérez et al., 2017). Taken as a whole, the study found that there were no significant differences in preference between iron-biofortified beans and conventional beans. The highest predictor of acceptance of the new beans was simply prior, general acceptance of beans. A clear point of the study was that nutritional information did not increase consumer preference for the high-iron beans in any instance. This seems to indicate that although nutritional information does not inform consumer preference for these crops, they can be widely accepted based on organoleptic qualities alone.

Over the last two decades there have been several developments in biofortification from a genetics and breeding perspective, however, the process requires a system-wide approach to be truly successful. Biofortification approaches require intimate cross-disciplinary

collaborations throughout the supply chain. For instance, plant breeders and agronomists are required to develop crop varieties that combine the best agronomic and nutritional traits; food scientists and nutritionists are required to screen bioavailability of nutrients, retention through cooking and processing, and human health impacts; policy researchers are required to establish regulations surrounding biofortified crops; economists are required to evaluate the financial impact of these crops; business managers are required to establish necessary infrastructure to allow for these crops to be successful; and finally grower education and know-how is essential in producing these crops (Nestel et al., 2006). Furthermore, Harjes points to the importance of intentionally nesting targeted breeding and genetic efforts within a socially acceptable context (Harjes et al., 2008). If placed in a socially acceptable context such that consumers will eat the biofortified crops, biofortification may be an exciting opportunity to enhance the nutritional qualities of raw materials.

2.7.2 Processing

Food processing can profoundly impact the nutritional content and quality of foods. Processing changes a raw material or ingredient into a finished product, often disrupting the food matrix of the original material. The food matrix is composed of the nutrients, bioactive compounds, and molecular bonds that form the structure, character, and attributes of a food (Aguilera, 2019). The food matrix determines a number of important factors, including digestibility, bioavailability, satiety, and glycemic response. Disrupting this matrix changes the health potential of a food. For example, a whole apple might have the same nutritional content as a serving of apple sauce, yet the glycemic response, satiety, and nutrient bioavailability differ between the two products (Fardet et al., 2018).

Similarly, breads made with flours of different particle sizes induce different glycemic responses (Fardet et al., 2018). Once more, the nutrient content is the same, but the different matrices, induced by different processing methods, produce different physiological responses.

Processing can also remove or replace nutrients, such as vitamins and minerals, from raw materials. For instance, grain milling, which strips away the bran and germ, removes fiber, antioxidants, minerals, and beneficial phenolic compounds (Heshe et al., 2016). In other cases, nutrients are added to foods through fortification and enrichment (Institute of Medicine (U.S.), 2003). For example, B vitamins, vitamin D, iron, and calcium are often added to common foods, like breakfast cereals (Nestle, 2020). Fortification and enrichment practices have been effective at curbing public health diseases, such as pellagra, indicating that certain processing techniques are effective at improving the nutritional quality of staple foods.

2.7.3 Policies influence health and supply chains

Policies drive the food system and play a major role in consumption and health outcomes. For example, policymakers enacted the Healthy, Hunger-Free Kids Act of 2010 (HHFKA) to improve nutrition in food served at schools throughout the United States. The bill set policy and authorized funding for several programs, including the National School Lunch Program (NSLP), the School Breakfast Program (SBP), the Special Supplemental Nutrition Program for Special Supplemental Nutrition Program for Women, Infants, and Children, the Summer Food Service Program, and the Child and Adult Care Food Program (USDA-FNS, 2013). The program enacted widespread

requirements that affected food and nutrient content in school meals, types of foods students need to select for meals to be eligible for federal reimbursement, meal prices, and types of foods and beverages sold during the school day (Fox & Gearan, 2019). The HHFKA played a major role in influencing the diet of school-age children. In a study comparing the nutritional quality of meals served prior to the Act to those served after the Act, researchers found a significant impact on nutritional quality of school meals. The nutrition quality of NSLP lunches improved between SY 2009-2010 and SY 2014-2015 based on mean scores of the Healthy Eating Index (HEI)-2010 (Fox & Gearan, 2019). During this time frame, the mean total HEI-2010 score increased from 57.9-81.5 out of a possible 100, indicating the updated nutrition standards significantly improved school lunch nutrition quality. There were also improvements in meeting food group and nutrient requirements without exceeding calorie limits, with the largest improvements occurring in the whole grain, greens, and beans categories. Whole grains increased from 25 to 95 percent of the maximum score while greens and beans increased from 21 to 72 percent between SY 2009-2010 and SY 2014-2015 (Fox & Gearan, 2019). Concentration of refined grains, empty calories, and sodium decreased in lunches served through NSLP during this time frame (Fox & Gearan, 2019).

Policies also influence supply chain performance. For instance, the HHFKA required increased whole grains offered at school lunch. Therefore, the whole grain supply chain responded to increased whole grain availability. As a result, the grain industry shifted to produce and deliver more whole grains to supply the demand of the National School Lunch Program. Indeed, the entire supply chain – plant geneticists and breeders, growers,

millers, government bodies, manufacturers, bakers, school food buyer groups and distributors, and school food service personnel – made a coordinated effort to facilitate the smooth translation of policy into transformational nutrition outcomes (Hauge et al., 2015).

2.7.4 Consumer integration

Consumer demand plays a critical role in value chain development. A 2013 study by Hattersly analyzed the canned fruit sector of the Australian agri-food system (Hattersley, 2013). Using a nutrition-oriented value chain research approach, the study aimed to identify key drivers and dynamics of the Australian agri-food system from a nutrition perspective. Unlike conventional value chain analyses, this paper focused on identifying implications for end-consumption rather than economic outcomes within the chain. Hattersly notes that major nutrition-related product innovation was historically consumer driven. For instance, over the last forty years the processed fruit sector shifted from preserving fruit in sugar syrup to preserving fruit in 100% fruit juice due to consumer demand. While this had a positive nutritional impact, more recent innovations have failed to address nutrition and health needs. Rather than innovating to create new, nutritionally sound products, most innovations in this sector over the past twenty years have focused on packaging and labeling. Furthermore, Hattersly notes that this system has focused on driving consumer demand rather than responding to it. Indeed, this system aims to expand markets by proliferating new food packaged options aimed at increasing consumer eating-occasions. Meanwhile, there is a gap between food and nutrition policy and the institutional framework that maintains the status quo of value addition via

innovations in packaging, labeling, and new product proliferation (Roseman et al., 2013; Scrinis, 2016).

Often, studies fail to adequately address consumer health outcomes, implications of the food environment, or the integration of the consumer voices throughout the value chain (Hattersley, 2013). Pelupessy and van Kempen note that most commodity chain studies to date have done a poor job at integrating consumers (Pelupessy & Kempen, 2005).

Intimately integrating consumers along each part of the supply chain could help companies develop infrastructure that more closely meets consumer needs. This efficient infrastructure could support nutrition-value-added products that more effectively address health outcomes.

2.7.5 Nutrition Sensitive Value Chain Approaches in the Developing World

Nutrition sensitive value chain approaches aim to integrate nutrition objectives into the value chain without compromising the economic value of the food (Summer Allen & Brauw, 2018). These approaches take into account the nutrition needs of the various actors throughout the supply chain, particularly the consumers. Beyond target population and quality (value) of the food, factors to consider in nutrition sensitive value chain approaches include nutrients of interest, vehicles that deliver nutrition (i.e. the food), bioavailability of the nutrient, interactions with other foods in the diet and the system, availability, acceptability, and cost. Additionally, supply and demand must be considered as the system needs to support production and consumption of enough product (at-scale) to improve nutritional status in a sustainable manner. This integrated approach also requires a significant behavioral change communication strategy in order to educate

consumers (Le Port et al., 2017). Due to the comprehensive, complex nature of this approach, there is limited research describing these interventions. However, recent studies suggest that a nutrition sensitive supply chain intervention coupled with behavioral change communication strategies can yield improvements in nutrition status.

Some research organizations have implemented nutrition sensitive value chain approaches in developing countries that are highly susceptible to micronutrient deficiencies. For example, a 2017 study by Le Port and colleagues used a supply chain approach that delivered micronutrient-fortified yogurt to families in Senegal, which ultimately improved iron status among children 24-59 months of age in Senegal (Le Port et al., 2017). Based on a contract and in addition to their standard pay, milk-producing Senegalese households received a daily micronutrient-fortified yogurt, under the condition that they delivered a standard quota of milk to a local dairy factory. Yogurt given in this study was made from milk produced in the region, and it was fortified with several shortfall nutrients like iron, iodine, zinc, and vitamin A. The group receiving the yogurt (the intervention group) was compared to a control group that did not receive the micronutrient-fortified yogurt. In parallel, a behavior communication campaign was developed to increase nutrition knowledge. Ultimately, this study showed that children from households that fulfilled the contract and received micronutrient-fortified yogurt experienced improvements in iron status compared to the control (Le Port et al., 2017).

A 2012 paper by Hotz and colleagues describes an integrative supply chain approach to increase vitamin A intake in Mozambique (Hotz et al., 2012). This study leveraged

farming/production practices to increase intake of a vitamin A biofortified orange sweet potato. Children between the ages of 6-35 months and their mothers or female caretakers were targeted through an intervention that consisted of three major components: agriculture, demand creation/behavior change communication, and product development/marketing. The agricultural component consisted of crop distribution, education, and training. Orange sweet potato vines were distributed to farmers over a three-year period, and researchers trained local farmers on best practices for managing the crop including pest management, harvest, environmental considerations. The demand creation/behavior change component aimed to raise awareness around the crop and to provide nutrition-related education. Researchers held social gatherings including field days, theater plays, and special programs around the importance of vitamin A and the benefits of the orange sweet potato. The marketing and product development component targeted medium scale growers, traders, and business owners in the general area. This phase involved training orange sweet potato traders, developing rural and urban markets for orange sweet potatoes, and developing specific, distinct market stalls focused on selling the product. Ultimately, this study demonstrated that those targeted by the intervention consumed more vitamin A biofortified orange sweet potatoes compared to the control group (Hotz et al., 2012). In fact, researchers observed between 42% and 169% increases in vitamin A consumption in children in the intervention group compared to the control. This study demonstrates that a comprehensive, whole supply chain approach can be leveraged to increase intake of nutrient dense foods.

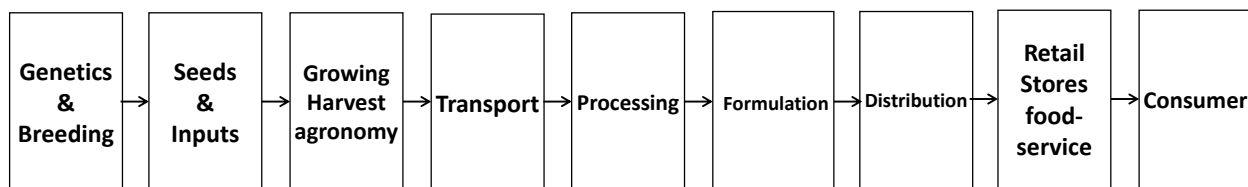


Figure 2.2 - Overview of a hypothetical supply chain

2.7.6 Opportunities for nutrition sensitive value chain approaches

The nutrition sensitive value chain approach has been implemented primarily in the developing world, with very few examples of scaling this approach in higher income countries. Adopting these concepts may be essential for improving the nutritional quality and sustainability of the supply chain. These concepts might be demonstrated by improving supply chains of current food sources or by developing new, sustainable food crops. Whole grains and pennycress provide examples of potentially sustainable food sources that also contribute to health. The following sections review some of the health, sustainability, and supply chain development opportunities of these whole grains and pennycress.

2.8 Whole grains and health: healthful, sustainable seed-based foods

Grains provide the foundation for global diets. Grain-based foods supply 25-50% calories to the Western diet (FAO, 1995). Out of 50,000 edible plants throughout the world, three staple crops, rice, maize, and wheat, provide more than 60% of total energy intake across the globe (FAO, 1995). In addition to these crops, sorghum, millet, oats, and barley are commonly consumed. Table 2.1 compares 2019 global grain production, trade, and utilization values of wheat, coarse grains (corn, barley, sorghum, millet, rye, and oats),

and rice. Grain production provides major economic output. In the United States alone, wheat production alone generated \$8.8 billion in 2019 (USDA-NASS, 2020b). Minnesota grain (e.g. corn, wheat, oats, barley, and rye) production totaled over \$5 billion in 2019 (USDA-NASS, 2020a). Clearly, grains are dietary and economic staples.

Table 2.1 - Global grain production, trade, and utilization estimates

2019 Global Grain Production, Trade, and Utilization Estimates (million tons)			
Grain	Production	Trade	Utilization
Wheat	762.2	175.1	757.5
Coarse Grain	1448.1	203.7	1429.8
Rice	500.6	44.9	503.0

Despite recent consumer trends toward low-grain or grain free diets, dietary guidance recommends adequate grain consumption as part of a healthful diet. The 2015 Dietary Guidelines for Americans (DGA) recommends consumption of six ounce-equivalents of grains per day (US Department of Agriculture and US Department of Health and Human Services, 2015). For example, an ounce-equivalent might be a single slice of bread or a half-cup of cooked rice. The DGA suggests that half of these servings should be whole grains. Furthermore, as total grain intake meets or exceeds recommendations across most age/sex groups, the USDA further recommends shifting intake away from refined grain and toward whole grain (US Department of Agriculture and US Department of Health and Human Services, 2015). Despite these recommendations, consumers often face barriers in identifying whole grain food sources.

There are several definitions of whole grain ingredients and whole grain foods. Grains consist of three basic components: the germ, bran, and endosperm. The germ is the embryo of the seed while the endosperm is comprised mainly of starch, which the seed uses as an energy source during growth. The bran is the outer protective layer of the seed. The endosperm is the largest portion of the seed followed by the bran and germ (Whole Grains Council, n.d.). The American Association of Cereal Chemists offers the following definition for whole grains: “Whole grains shall consist of the intact, ground, cracked, or flaked caryopsis whose principal components, the starchy endosperm, germ, and bran, are present in the same relative proportions as they exist in the intact grain,” (American Association of Cereal Chemists International, 1999). Similarly, the Whole Grains Council specifies that whole grain ingredients and foods must contain all parts of the naturally occurring grain, the bran, germ, and endosperm, in the same relative proportions as they would appear in the intact grain regardless of processing while also supplying the same balance of nutrients found in the original caryopsis (Whole Grains Council, n.d.). Whole grains stand in contrast to refined grains, which lack the bran and germ, and consist entirely of the starchy endosperm. While the starchy endosperm contains mostly carbohydrates, the bran and germ are nutrient dense. These components contain important nutrients like fiber, vitamin E, magnesium, iron, and other beneficial phytochemicals (Rebello et al., 2014). Extracting the bran and germ reduces the nutrient quality of the grain. Common staples designated as whole grain by the USDA include: whole-wheat flour, bulgur, oatmeal, whole cornmeal, and brown rice (USDA, n.d.). Table 2.2 shows the nutrient content of several common grain-based foods and compares refined versus whole grain variants. In general, whole grain foods contain fewer calories,

more fiber, and more vitamins and minerals than their refined grain counterparts (Rebello et al., 2014). This increased nutrient content contributes to the health benefits of whole grain consumption.

Table 2.2. - Nutrient content of common grain-based foods (per 100 grams)

Nutrient Values of Whole Grain and Refined Grain Products (per 100 grams)						
Product	Energy (Kcal)	Protein (g)	Carbohydrate (g)	Fat (g)	Fiber (g)	Sugar (g)
Bread, whole wheat	252	12.45	42.71	3.5	6.0	4.34
Bread, white	266	8.85	49.42	3.33	2.7	5.67
Pasta, whole grain, cooked	148	5.95	29.89	1.7	3.9	0.75
Pasta, cooked	157	5.76	30.68	0.92	1.8	0.56
Muffin, whole grain	360	6.61	45.75	17.4	1.8	20.06
Muffin, plain	368	5.77	51.88	15.62	0.8	27.65

Nutrient composition varies across different grains. Therefore, physiological responses differ based on consumption of different grains. For instance, wheat and rye are both rich in insoluble fiber, which adds bulk to stool and aids in laxation (McRorie & McKeown, 2017). In contrast, oats and barley are rich in the soluble fiber beta-glucan. Consumption of beta-glucan is associated with improved cholesterol and glucose control (McRorie & McKeown, 2017). Given the diversity of nutrient composition, consuming a variety of whole grains can supply a wide range of nutrients and physiological benefits while improving dietary diversity.

Whole grains are associated with several health benefits, and they are widely regarded as a key component of a healthy lifestyle. As noted by O'Neil and colleagues, whole grain consumption is often associated with overall higher diet quality and nutrient intake (O'Neil et al., 2010). Whole grain consumption is also associated with other positive lifestyle factors such as exercise (Jr et al., 1998; Liu et al., 1999; O'Neil et al., 2010). Epidemiological and observational studies indicate that diets rich in whole grains are associated with decreased mortality and decreased risk for T2DM, cancer, and CVD (Ampatzoglou et al., 2015; Dagfinn Aune et al., 2016a; Brownlee et al., 2010; Parker et al., 2013). Aune and colleagues note that the nutrients, phytochemicals, and fiber in whole grains act synergistically to control insulin response and glucose control, thus protecting against T2DM (Dagfinn Aune et al., 2013). A study following 160,000 women over 18 years showed those who ate 2-3 servings of whole grains per day were 30% less likely to develop T2DM compared to those who rarely consumed whole grains (de Munter et al., 2007). This relationship is significant as T2DM is expected to impact 1 in 3 Americans by 2050 (CDC, 2010). Whole grain consumption is also inversely related to colorectal cancer, one of the leading causes of cancer-related deaths in the US (D. Aune et al., 2011; Strayer et al., 2007). Whole grains also protect against CVD. In fact, it was reported that women who eat 2-3 servings of whole grain per day are 30% less likely to die from heart disease over a 10-year period compared to those who ate less than 1 serving per week (Liu et al., 1999). Similarly, people who consume 2.5 or more servings of whole grain per day are 21% less likely to experience CVD compared to those who consume less than 2 servings per week (Mellen et al., 2008). The relationship between whole grains and CVD is important as cardiovascular disease is a leading cause of

mortality and healthcare expenditure in the US. Taken together these reports indicate that whole grain consumption can protect against some of the most critical non-communicable diet related diseases.

Whole grain production and consumption also contributes to environmental sustainability. Wells and Buzby suggest that increased whole grain consumption could improve land-use efficiency (Wells & Buzby, 2008). In their model, an equal amount of land produces more volume of whole grain product compared to refined grain product. This occurs because the entire kernel is used to produce whole grain products, whereas refined grain products lose 30-40% of the kernel. Clark and colleagues indicate that increased whole grain consumption has both health and environmental benefits compared to other food groups (M. A. Clark et al., 2019). Taken together, their health benefits and low environmental impacts position whole grains as a nutritious, sustainable food.

2.8.1 Whole grain consumption

Whole grain consumption falls short of DGA recommendations. As noted by Albertson and colleagues in a study examining whole grain consumption and body weight trends via NHANES 2001-2012 data, on average US adults consume 6.9 ounce equivalents of grains per day, of which 0.92 ounce equivalents are whole grains (Albertson et al., 2016). Though this study showed an increase in whole grain consumption from 2002-2012 (Albertson et al., 2016), actual consumption falls short of DGA recommendations. Whole grain consumption falls short in youth populations as well. The Institute of Medicine reports that children aged 5-8 consumed less than 24% of whole grain recommendations, while children aged 9-13 and 14-18 consumed less than 20% of whole grain

recommendations (Committee on Nutrition Standards for National School Lunch and Breakfast Programs & Institute of Medicine, 2010). This is particularly alarming as adolescent obesity has tripled since the 1970s, with recent reports indicating that 1 in 5 children aged 6-19 experienced obesity between 2015-2016 (Hales, 2017). Adolescent overweight and obesity are associated with increased risk of T2DM and metabolic syndrome and increased mortality in adults (Biro & Wien, 2010; Rush & Yan, 2017). Whole grain consumption is associated with improved diet quality and nutrient intake in adolescents (O'Neil et al., 2010).

Substituting whole grain for refined grain may subtly improve dietary intake by increasing the nutritive value of foods without drastically changing acceptability and organoleptic qualities of meals (Rosen et al., 2011). However, barriers exist within the food system and food environment that prevent the incorporation and increased availability of whole grain foods. These barriers include limited knowledge regarding whole grains, undesirable taste or texture qualities, increased preparation time, higher cost, and low availability in foodservice settings (Rosen et al., 2011).

More work is needed to understand barriers to whole grain consumption not only at the consumer level, but also addressing barriers, challenges and opportunities within the food supply, food environment and socio-cultural norms within diverse communities. In addition to health impacts, food and nutrition scientists ought to consider the other dimensions of the nutrition ecology framework (environmental, economic, and social)

when developing food solutions. This could potentially be achieved through nutrition-sensitive value chain approaches.

2.9 Field pennycress: a sustainable crop with potential food use applications supporting the sustainable, nutritional intensification of agriculture

New crop development provides another opportunity to implement nutrition sensitive value chain approaches. For example, the University of Minnesota is developing a portfolio of new crops that contribute to environmental sustainability via ecosystem services. There may be opportunities to work throughout the value chain to improve the nutritional qualities of these crops. Field pennycress provides one example of this type of crop.

Field pennycress (*Thlaspi arvense L.*, pennycress herein) is a winter annual oilseed undergoing development as a cash cover crop for the Upper Midwest. As a winter annual, this crop is planted in the fall and matures in the early spring. Pennycress is particularly cold tolerant and winter hardy. These qualities allow pennycress to fit within conventional crop systems, such as the corn-soybean rotation, which dominates much of the Upper Midwest landscape.

Figure 2.3 illustrates a potential corn-pennycress-soybean crop rotation. In this model, pennycress is planted prior to (e.g. inter-seeded into standing corn, i.e. relay cropped) or immediately following a fall corn harvest (Johnson et al., 2015; Phippen & Phippen, 2012). Once the crop matures in the spring, pennycress is harvested prior to planting soybeans. As a result, pennycress improves the biodiversity of crop production by

providing a living cover to landscapes that are traditionally left barren during the winter fallow period. During this timeframe, pennycress provides several ecosystem services and potential environmental benefits (Schipanski et al., 2014). For instance, the pennycress prevents nitrate leaching and soil erosion, which has a downstream benefit of protecting against water pollution (Weyers et al., 2019). This is an important feature as nitrate leaching contributes to hypoxia and dead zones in critical water sources (The United States Environmental Protection Agency [EPA], 2020). Winter cover crops, like pennycress, also sequesters key soil nutrients, such as carbon, phosphorus, and nitrogen, improving soil quality and health (Dabney et al., 2001). This potentially reduces the use of fertilizers, which, when used in excess, can cause deleterious environmental effects (Smith et al., 2007). Additionally, improved soil quality is beneficial for following crops, like soybean (Stahl et al., 2016). Pennycress also suppresses establishment of weeds and provides a food source for spring pollinators, like bees (Eberle et al., 2015). Taken together, these environmental factors may yield downstream supply chain benefits, such as reduced input use, reduced costs for producers, increased consumer interests, and increased corporate social responsibility opportunities and benefits for food companies.

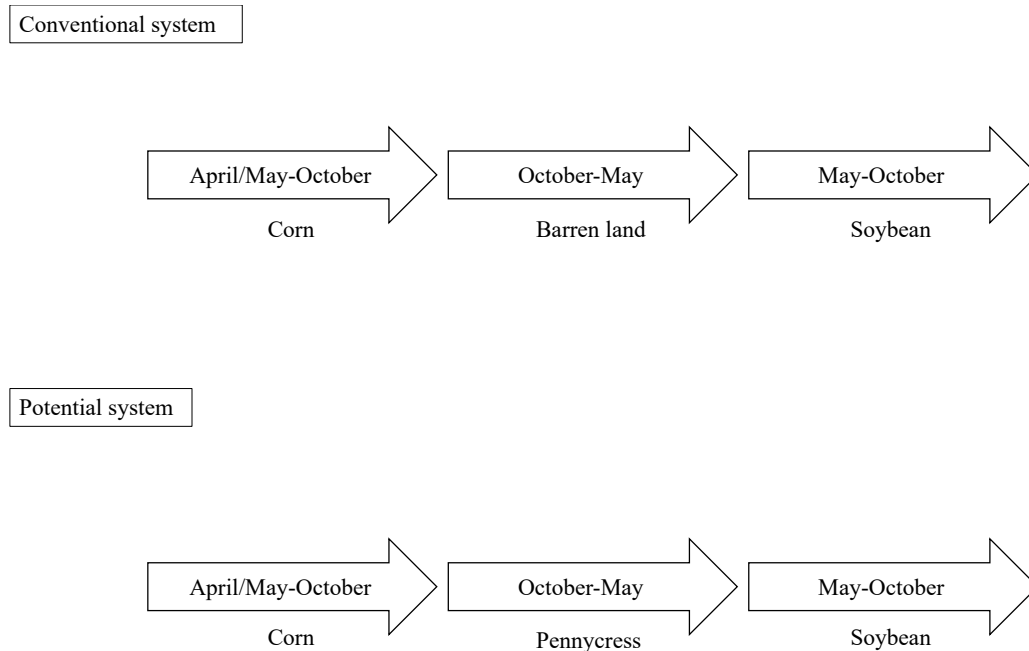


Figure 2.3 - Potential corn-pennycress-soybean crop rotation

Current work aims to optimize the pennycress system using a multiple-disciplinary approach. This multiple-disciplinary approach spans the entire supply chain (i.e. pre-farm → on-farm → post-farm → consumer) and includes genetic, agronomic, economic, processing, food science, and regulatory components. For instance, agronomic work aims to identify farming practices and crop rotations that best suit pennycress deployment along with the environmental impacts of including the crop in conventional systems. Johnson and colleagues explored the yield tradeoffs and nitrogen impacts of relay- and double- cropping pennycress and winter camelina with soybean (Johnson et al., 2017). They found that, while the winter cover crops may reduce soybean yield, they greatly decrease the amount of extractable inorganic soil nitrogen, particularly in the Spring and Autumn (Johnson et al., 2017). Ott and colleagues found that relay-cropping pennycress with soybeans resulted in a 30% yield reduction in soybeans (Ott et al., 2019). However,

total oilseed output (i.e. pennycress + soybean) was equivalent or greater than mono-cropped soybean (Ott et al., 2019). In many different crop treatments, income was equal to or slightly less than mono-cropped soybean, potentially due to increased fertilizer costs of producing the extra crop. Moore and colleagues examined the sustainability implications of pennycress production in sweet corn systems, finding that pennycress produced stable yields without supplemental fertilizer and reduced residual nitrogen throughout the system (Moore et al., 2020). In some cases, combining pennycress within conventional crop rotations (corn-soy) can improve total oilseed output compared to rotations lacking pennycress (Hoerning et al., 2020; Ott et al., 2019). While many of these studies show that pennycress exhibits a wide range of yield across environments, current work aims to improve the plant to produce consistent yields as part of the domestication process.

Rapid advances in genomic and genetics tools have facilitated the discovery of many traits crucial for crop development. Researchers at the University of Minnesota assembled a draft pennycress transcriptome in 2013 and a draft pennycress genome in 2015 (K. M. Dorn et al., 2015; Kevin M. Dorn et al., 2013). Research teams from the University of Minnesota, Western Illinois University, and Illinois State University developed a strategy to target key domestication traits (Sedbrook et al., 2014). In brief, this included the tenets of the domestication syndrome, including reduced seed dormancy, reduced seed dispersion (i.e. shatter), increased seed size, apical dominance, uniform size, and uniform flowering along with improved seed meal and oil quality traits (Sedbrook et al., 2014). These traits are critical to transforming the plant from a weedy

species to an agronomically valuable crop. For instance, a study by Cubins and colleagues found that seed shatter alone accounted for up to 70% yield loss, potentially reducing the economic vitality of the system (Julija Alda Cubins, 2019). Chopra and colleagues identified several key domestication traits and devised a strategy to stack these traits into a developed pennycress line, similar to canola (Chopra et al., 2020). Importantly, improving oil and seed meal quality are essential targets for unlocking end use, and thus market potential for pennycress.

Oil content in oilseeds such as soybean, canola, and flax ranges from ~20-45% oil. Similarly, pennycress produces oil-rich seeds, ranging from 26-39% oil (Moser, Shah, et al., 2009). Given its high oil content, many efforts aim to develop pennycress oil for bio-based applications, such as biodiesel, industrial lubricants, and plastics. The pennycress oil profile consists of erucic acid (~35%), linoleic acid (20%), linolenic acid (13%), oleic acid (11%), and eicosenoic acid (10%) (Chopra et al., 2019, 2020; Evangelista et al., 2012; Moser, Knothe, et al., 2009; Moser, Shah, et al., 2009). This fatty acid profile confers several properties, such as high viscosity and cold flow, that make pennycress an attractive candidate for industrial uses, such as industrial lubrication and aviation fuel (Moser, Knothe, et al., 2009; Moser, Shah, et al., 2009). Some studies have performed life cycle analyses (LCAs) and techno-economic assessments (TEAs) to evaluate the potential impact of pennycress production on biofuel.

2.9.1 Lifecycle analyses and techno-economic assessments of pennycress production

Given its potential sustainability impact and suitability for the biofuel market, several studies aim to elucidate the environmental and economic implications of pennycress

production. Pennycress has the potential to be a renewable source of fuel (Moser, Knothe, et al., 2009). This is critical as the United States is heavily reliant on non-renewable fossil fuels, particularly petroleum. In 2018, the United States consumed 20.5 million barrels of petroleum per day (United States Energy Information Administration, 2019). The transportation and industrial sectors use 4.16 and 5.13 barrels per day, respectively. This accounts for nearly 806 million gallons of petroleum. Jet fuel alone uses about 1.71 million barrels per day, making it the fourth largest petroleum-based product. Pennycress is considered an attractive bio-based jet fuel candidate, given its high oil content and suitable oil profile, which is high in unsaturated fatty acids (Moser, Shah, et al., 2009). More work is needed to understand potential production implications of pennycress production at scale, and recent studies aim to understand the economic and environmental outcomes associated with this system.

Life cycle assessments (LCAs), sometimes referred to as cradle-to-grave analyses, are tools used to evaluate the potential environmental impacts of a product from raw material production to material acquisition, processing, manufacturing, use, and final disposition (EPA, 2017). Fan and colleagues (2013) conducted an LCA to illustrate the potential environmental implications of using pennycress as a feedstock for biofuel production (J. Fan et al., 2013). Here, researchers found that life cycle greenhouse gas emissions of pennycress-derived renewable diesel and hydro-processed renewable jet fuel were 50% of those produced by petroleum-based counterparts (J. Fan et al., 2013). The potential for decreased greenhouse gas emissions via pennycress derived renewable biofuels could be critical for reducing transportation sector greenhouse gas emissions, 29% of all

greenhouse gas emissions and energy use (EPA, 2020). While there appears to be clear environmental benefits of producing pennycress-derived renewable biofuels, both on farm and post-harvest, the system must remain technically and economically feasible. Techno-economic assessments (TEAs) provide a framework to evaluate the technological and economic feasibility of a process. This framework merges process modeling, engineering design, and economic evaluation by harnessing data from a wide variety of sources to illustrate potential economic viability and identify process bottlenecks (ABPDU, 2020). Mousavi-Avval and Shah (2020) performed a TEA to evaluate the pennycress production, harvest, and post-harvest logistics for renewable jet fuel (Mousavi-Avval & Shah, 2020). Here, the researchers mapped the production system including land use, equipment and machinery needs, labor requirements, consumables, post-harvest logistics, and commercial scale manufacturing activities for an Ohio-based biorefinery (Mousavi-Avval & Shah, 2020). They projected that 41-63 thousand hectares with an annual harvest of 90-115 thousand tons are required to supply a single biorefinery (Mousavi-Avval & Shah, 2020). Total production costs ranged from 170-230 dollars per ton, which is considerably less than comparable oilseeds, like canola, camelina, and carinata (Mousavi-Avval & Shah, 2020). Up to this point, pennycress LCAs and TEAs make critical assumptions about the potential supply chain. These assumptions are based on the idea that this crop will most likely fit into a supply chain similar to other oilseeds, like canola in particular. Notably, Fan and colleagues and Hashem and Shah both assume that pennycress meal is suitable for animal consumption (J. Fan et al., 2013; Mousavi-Avval & Shah, 2020). This indicates that improving meal quality is essential for pennycress development, thus valorizing a major waste stream of the crushing process.

Regardless of the meal, it is clear there are opportunities for industrial use of pennycress oil.

Beyond industrial applications, pennycress oil and meal have garnered interest for food use, and the following sections focus on oil and meal characteristics and barriers related to food use. More work is needed to assess the potential supply chain for a food-grade pennycress, including genetic mechanisms underlying production of toxic compounds and production economics of the pennycress system. The following sections briefly describe key considerations for oil quality. Then meal quality is discussed, with a specific emphasis on glucosinolates, followed by a brief discussion of other anti-nutritional components. The oil and meal quality discussions briefly highlight recent genetic improvements meant to facilitate the development of food grade pennycress. Finally, the canola supply chain is discussed to provide historical and techno-economic context for a potential pennycress supply chain.

2.9.3 Oil Quality – Considerations for Food Use

Improving oil quality is critical to unlocking the food use potential of pennycress. Similar to rapeseed, wild pennycress is rich in erucic acid, making it unfit for human consumption. High doses of erucic acid are associated with heart disease in animal models (Hulan et al., 1976). Given this potential toxicity, the maximum level of erucic acid allowed in food oil is set at 2% by weight in the United States (21CFR184.1555). In contrast, wild pennycress contains 35% erucic acid. Recently, low-erucic acid mutant pennycress lines have been identified (Chopra et al., 2020). These plants harbor a

mutation in the FATTY ACID ELONGATION1 (FAE1) gene (Chopra et al., 2020), which, over the course of two steps, converts oleic acid to erucic acid. The *fae1* mutation results in a reduction of erucic acid to canola-like levels. However, the *fae1* mutants also exhibited increased levels of the polyunsaturated fatty acids (PUFAs) linoleic acid and linolenic acid (Chopra et al., 2020). While PUFAs are beneficial fatty acids, associated with health benefits like reduced risk for heart disease, they render the oil less stable (Finley & Shahidi, 2001). Oils rich in PUFAs oxidize more quickly and produce off-flavors and aromas, making them less suitable for product formulation. Chopra and colleagues (2020) identified a mutation in the REDUCED OLEATE DESATURATION 1 (ROD1) gene, which resulted in a reduction in both linoleic and linolenic acid. Combining the FAE1 and ROD1 mutations resulted in a pennycress line with an oil profile comparable to canola (Chopra et al., 2020). In addition to the reduction in erucic acid and the linoleic, and linolenic acid, this combination also results in an increased oleic acid content, which is beneficial for oil quality.

Oleic acid is a monounsaturated fatty acid found in many food oils. Increased oleic acid content is desirable in many food applications. For instance, higher oleic acid content results in a higher smoke point, i.e. the temperature at which an oil starts to burn or produce smoke (Canola Council of Canada, n.d.). High smoke points are desired in frying applications as the oil can be heated to a higher temperature for longer periods of time. These oils tend to be more stable with longer shelf lives. Taken together, these attributes increase frying efficiency and reduce waste. In addition to frying, oleic acid-rich oils are also used in baking, sautéing, and as a base in salad dressings. High oleic oils have been

used to replace partially hydrogenated oils, which tend to contain high levels of trans fats, in many foods. Trans fats are associated with increased LDL-cholesterol (i.e. “bad” cholesterol) and decreased HDL-cholesterol (i.e. “good cholesterol), which increases risk of heart disease (McNamara, 2014). Trans fats were banned by the FDA in the mid-2000s and are now nearly absent from the food supply (Unnevehr & Jagmanaite, 2008). This regulation has the potential to increase market demand for high oleic oils.

Oleic acid has garnered attention for its potential health benefits. Some evidence supports that oleic acid provides cardiovascular benefits when it replaces saturated fatty acids (Voelker, 2019). In 2018, food oils with increased oleic acid content (at least 70% oleic acid) received a qualified health claim by the United States Food and Drug Administration (FDA), allowing companies producing high oleic oils to label products as heart healthy (Gottlieb, 2018). Qualified health claims, which indicate that some evidence supports the health impact of a product, are considered less robust than authorized health claims, which are backed by all publicly available scientific evidence (Voelker, 2019). Label health claims are partially meant to incentivize the food industry to reformulate products (Gottlieb, 2018). As a result, many companies now tout their high oleic acid food oils. Cargill, one of the largest privately-owned companies in the world, markets conventional and organic high oleic sunflower and canola oils (*High Oleic Oils*, 2020). Calyxt, an emerging crop biotechnology company, markets a high oleic soybean oil (*Healthier, More Sustainable Ingredients*, 2020). Both companies emphasize the health claim potential of their oils in addition to their functional qualities. These oils are generally sold to commercial scale frying companies or food service operations.

2.9.4 Seed Meal Quality

Improving meal quality is critical to unlocking the food use potential for pennycress. Furthermore, improving meal quality valorizes the major waste stream of the crushing process, thus improving the value of the overall pennycress system, regardless of industrial or food use. Thousands of pounds of seed meal (i.e. press-cake) are generated as a waste stream from the oilseed crushing process. This seed meal must be utilized to ensure the economic viability of the process. Pennycress is rich in protein, with seeds containing 25-27% protein (Warwick et al., 2002). Given its rich protein content, pennycress seed meal could be positioned as an animal feed additive or, potentially, a plant-based protein source for human food, similar to bi-products of comparable oilseeds. However, negative compounds must be reduced to fully utilize this seed meal.

Wild pennycress contains several components, like fiber, glucosinolates, and tannins, that limit utility in animal feed and human food applications. Reducing these compounds is a key step in improving the value of pennycress, as demonstrated by other oilseeds. For example, glucosinolate reduction was a key target in developing canola (J. M. Bell, 1993).

Glucosinolates are sulfur-rich secondary metabolites found in plants belonging to the Brassicaceae family. These compounds, along with their breakdown products, contribute to the sharp, pungent odors and flavors associated with these crops, like mustard and horseradish (Martinez-Ballesta & Carvajal, 2015). In fact, glucosinolates, and their breakdown products, contribute to the lay nickname for pennycress, “stinkweed.”

Glucosinolates are a diverse class of compounds, with over 200 different known glucosinolates. These compounds are classified as aliphatic, aromatic, or indole based on their structure (Halkier & Gershenzon, 2006). Glucosinolate biosynthesis has been well-characterized in the model plant *Arabidopsis* and other plants in the Brassicaceae (Halkier & Du, 1997; Halkier & Gershenzon, 2006; Ida E. Sønderby et al., 2010). Figure 2.4 provides an overview of the glucosinolate biosynthesis pathway. Briefly, glucosinolate biosynthesis occurs in three phases mediated by several different enzymes and regulated by several different transcription factors, with crosstalk between other important plant pathways (Halkier & Gershenzon, 2006). First, an amino acid undergoes side chain elongation followed by core structure biosynthesis. Then the core structure undergoes a series of secondary side chain elongation reactions. This phase results in the diversity of the compound. Many different glucosinolates can occur within a single plant. **Table 2.3** lists several vegetables and agronomically important crops belonging to the Brassicaceae and includes common glucosinolates found in each crop.

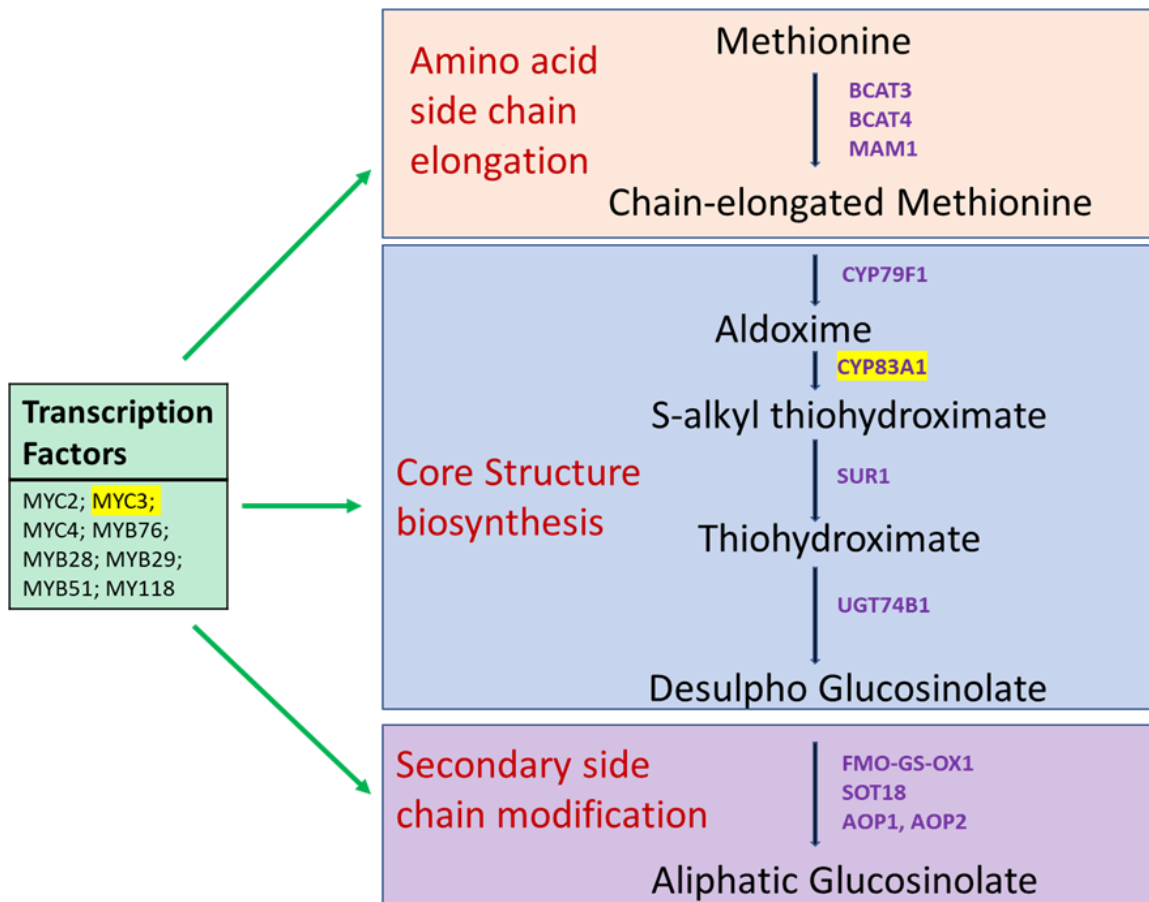


Figure 2.4 – Glucosinolate biosynthesis pathway

Table 2.3 - Common Glucosinolates and breakdown products in brassicas

Food Source	Glucosinolate	Breakdown Product
Broccoli	Glucoraphanin	Sulforaphane
Cabbage	Glucotropaeolin	Benzyl-Isothiocyanate
Cauliflower	Glucobrassicin	Indole-3-Carbinol
Mustard	Sinigrin	Allyl-Isothiocyanate

Glucosinolates work in concert with membrane bound thioglucosidase enzymes, called myrosinases, to defend against pests (Bhat & Vyas, 2019; Fenwick & Heaney, 1983). Myrosinases are physically separated from glucosinolates in plant tissues. Once tissues are damaged, such as via cutting or chewing, myrosinases interact with glucosinolates.

The enzymes hydrolyze glucosinolates to generate highly reactive breakdown products, such as thiocyanates, isothiocyanates, indoles, oxazolidine-2-thiones, epithionitrile, and nitrile, which fend off pests (Martinez-Ballesta & Carvajal, 2015). Myrosinase enzymes can be degraded using high heat, which renders them inactive; however, bacterial myrosinase-like activity in the colon stimulates glucosinolate hydrolysis and produces a wide range of breakdown products (Ishida et al., 2014). This bacterial mediated hydrolysis is part of the internal detoxification system, which is responsible for processing glucosinolates in the body. Table 2.3 lists common breakdown products associated with glucosinolates in various brassicas.

Glucosinolates and their breakdown products can be deleterious when consumed in high amounts. Given their pungent aroma and sharp, bitter flavor, glucosinolates, when present in high amounts, can render seed meal unpalatable, which reduces intake and impairs animal growth (Tripathi & Mishra, 2007). Additionally, glucosinolate breakdown products can interfere with iodine uptake, which impairs production of the two thyroid hormones triiodothyronine and thyroxine (Tripathi & Mishra, 2007). This disruption in iodine metabolism can lead to hypothyroidism and enlargement of the thyroid gland, i.e. goiter. Impaired thyroid function and the resulting toxicity can cause other negative downstream impacts, including decreased growth, fertility, milk production, egg production, liver function, and kidney function (European Food Safety Authority (EFSA), 2008). Additional reports indicate that chickens fed high-glucosinolate feeds produce eggs with a distinct fishy odor (Khajali & Slominski, 2012; Tripathi & Mishra,

2007). Taken together, these negative impacts have limited the use of brassica-based meal as a protein supplement in animal feed.

While feeding studies in animal models show the negative implications of glucosinolate consumption, human-based epidemiological evidence is less conclusive. Some literature suggests that glucosinolates and their breakdown products offer chemoprotective benefits while others suggest that high glucosinolate consumption increases risk for chronic disease such as type 2 diabetes and heart disease. Retrospective studies indicate glucosinolate consumption is linked with decreased risk of cancers, including lung, colorectal, breast, and prostate cancers (Kopjar et al., 2012; Prieto et al., 2019; Traka, 2016; Verkerk et al., 2009). This chemoprotection is potentially the result of glucosinolate breakdown products stimulating the xenobiotic detoxification system. For instance, isothiocyanates activate glutathione-S-transferases, UDP-glucuronosyl transferases (UGTs), NADPH quinone oxidoreductase (NQO), and glutamate cysteine ligase, which protect against DNA damage, reactive oxygen species, and carcinogens (Kensler et al., 2004; Yuesheng Zhang, 2004). More work is needed to better understand potential mechanisms of protection.

Recent prospective cohort studies indicate that higher glucosinolate consumption may be associated with increased risk for type 2 diabetes and heart disease. In one prospective study following health professionals over several years, individuals with the highest glucosinolate intake had a 19% increased risk of type 2 diabetes compared with those with the lowest intake after adjusting for other potential risk factors (Ma, Liu, Sampson,

et al., 2018). High glucosinolate consumers also had higher risk for heart disease compared to low glucosinolate consumers (Ma et al., 2018). In both cases, there appeared to be a particularly strong association with Brussels sprout consumption. More work is needed to understand the potential toxic or chemoprotective effects of glucosinolates in humans. Since cruciferous vegetables also contain other beneficial bio-active compounds, glucosinolates may function in concert with these nutrients to produce synergistic chemoprotection. Additionally, future studies may try to elucidate the health impact of specific glucosinolates – e.g. sinigrin vs glucoraphanin – which may be difficult as these glucosinolates can occur in the same food source.

The potentially toxic effects of specific glucosinolates are often hard to quantify given many glucosinolates can occur in a single plant. Therefore, regulatory standards are based on total glucosinolate content rather than individual glucosinolate content (European Food Safety Authority (EFSA), 2008). Wild pennycress contains 100 $\mu\text{mol/g}$ glucosinolates, which is similar to rapeseed, which contains 110-150 $\mu\text{mol/g}$ glucosinolates (J. M. Bell et al., 1991). In contrast, canola contains \sim 4-15 $\mu\text{mol/g}$ glucosinolates, with the standard set at <30 $\mu\text{mol/g}$ (European Food Safety Authority (EFSA), 2008). Pennycress glucosinolates occur predominantly in the form of the aliphatic glucosinolate sinigrin (Warwick et al., 2002). Reducing pennycress glucosinolates to levels below the canola quality threshold has been a key focus of pennycress development (K. M. Dorn et al., 2015; Sedbrook et al., 2014). In *Arabidopsis* and other brassicas, several genetic targets conferring a low glucosinolate phenotype have been identified (Ida E. Sønderby et al., 2010). For example, reducing the function of the

transcription factor MYB28, also called High-Aliphatic Glucosinolate 1 (HAG1), and the glucosinolate transporters GTR1 and GTR2 reduces glucosinolate accumulation (Nour-Eldin et al., 2012, 2017). Recently, Chopra and colleagues identified a pennycress line harboring a mutation in the secondary side chain modification gene *ALKENYL HYDROXALKYL PRODUCING 2 (AOP2)*, with a glucosinolate concentration <20 $\mu\text{mol/g}$ (Chopra et al., 2020). Seeds from this mutant are reported to have a pleasant, nutty flavor, while the leaves reportedly lack the garlicky aroma associated with wild pennycress (Chopra et al., 2020). More work is required to understand the potential relationship between reduced glucosinolates and the pleasant flavor and aroma of the *aop2* mutant. A recent report indicates that altering glucosinolates influences amino acid profiles and other seed characteristics (Slaten et al., 2020). Perhaps reducing glucosinolates in pennycress could yield other positive characteristics resulting from a shift in amino acid metabolism. Regardless, it is clear combining the low glucosinolate trait along with other beneficial traits could produce a commercially viable pennycress variety.

Reducing glucosinolates could produce unintended consequences. Glucosinolate reductions can influence plant fitness and increase susceptibility to pests. This may require increased pesticide applications, which could increase input costs for farmers. Additionally, increased pesticide and chemical application could yield negative environmental implications. Targeting the glucosinolate transporters GTR1 and GTR2 may offer a solution for this problem. GTR1 and GTR2 transport glucosinolates into the seed (Nour-Eldin et al., 2012, 2017). Studies in *Arabidopsis* show that knocking out

GTR1 and GTR2 reduces seed glucosinolates, but glucosinolate concentrations are maintained throughout other plant tissues. In other words, knocking out these transporters has the potential to produce a higher value seed without sacrificing the protection against pests. While reducing glucosinolates is an essential target for food use, some reports suggest high-glucosinolate brassica meal is an effective biofumigant (Vaughn et al., 2005). Vaughn and colleagues report that glucosinolate rich pennycress meal significantly suppresses weeds in field studies (Vaughn et al., 2005). This property may position pennycress seed meal as an attractive candidate for use as a natural herbicide in organic or conventional crop systems.

Similar to canola, pennycress may contain other components, such as fiber, condensed tannins, phytic acid, and sinapine which can limit seed meal value. Defatted pennycress seed meal has been reported to contain 15% crude fiber (Hojilla-Evangelista et al., 2015). Increased fiber content is associated with decreased available energy and metabolizable energy in other brassica seed meals, which can limit animal growth (J. M. Bell et al., 1991; Mejicanos et al., 2016). Additionally, the dark pennycress seed coat contains condensed tannins, which potentially limit seed utility. Condensed tannins have been shown to inhibit iron absorption and contribute to astringency in canola (Naczek et al., 2000). Chopra and colleagues have identified mutants with lighter seed coats containing decreased condensed tannins (Chopra et al., 2020). Phytic acid may also decrease seed meal value. This compound, found in many seeds, including canola and whole grains, is associated with impaired zinc, iron, calcium, and magnesium absorption (Gupta et al., 2015). Notably, these are shortfall nutrients around the globe, and diets high in phytic

acid are associated with micronutrient deficiency (Wessells & Brown, 2012). Sinapine, a choline ester of sinapic acid, is another potential anti-nutrient present in pennycress. This compound confers a bitter flavor to animal feeds, which limits palatability and animal growth (J. M. Bell, 1993; Mailer et al., 2008; Mejicanos et al., 2016). Additionally, chickens fed diets high in sinapine have been reported to lay eggs with a fishy odor and flavor (J. M. Bell, 1993; Shahidi & Naczki, 1992). While glucosinolates are the clear near-term target to improve pennycress for food use, addressing these other antinutritive components may add further value to pennycress seed meal. Reducing these anti-nutrients can improve nutritional quality of seed meal and reduce potential negative sensory characteristics associated with the compounds. Notably, Chopra and colleagues have created a mutant gene database for pennycress, which has facilitated the discovery of genes conferring added value to pennycress (Chopra et al., 2018, 2020). For instance, the mutant pennycress lines with lighter seed coats have multi-pronged value for the potential supply chain: 1) the seed coats lack antinutrients and 2) also thinner seed coats can improve crushing efficiency.

2.9.5 Regulations – GRAS

Pennycress oil and seed meal must meet key regulatory requirements to fit into the food oil and meal supply chains. Notably, pennycress oil and meal lack FDA Generally Recognized As Safe (GRAS) status. According to the FDA:

“[A]ny substance that is intentionally added to food is a food additive, that is subject to premarket review and approval by FDA, unless the substance is generally recognized, among qualified experts, as having been adequately shown

to be safe under the conditions of its intended use, or unless the use of the substance is otherwise excepted from the definition of a food additive,” (FDA, 2019).

In summary, using a food ingredient in a product requires a general recognition of safety for the intended use of the ingredient. Ingredient safety must be supported by a substantial quantity of quality scientific research, and safety must be agreed upon by a panel of qualified experts.

Generally, food and ingredient companies (designated as the notifier) coordinate with external consultants to assemble a dossier containing data supporting the safety of a specific additive or ingredient. Table 2.4 outlines some of the key components of the dossier, as recommended by the FDA. The dossier includes the identity of the substance, including chemical structure, composition, and characteristic properties. For biological substances, this includes taxonomic and sub-species data, part of the plant that produces the substance, and any potential toxic components (FDA, 21CFR170.230). The dossier also requires a thorough description of manufacturing processes and specifications for food grade materials (FDA, 21CFR170.230). Most importantly, notifiers must include data supporting the safety of the substance, including information related to the physical effect of the substance on a consumer, potential negative impacts of consumption, and levels at which a substance might be dangerous (FDA, 21CFR170.230). For example, notifiers might include feeding studies (in either animal or human models) describing the level of inclusion at which there are no negative effects, also called the no observable

adverse effects level. Notifiers are also required to submit dietary exposure information, including the amount consumers are likely to ingest as part of a total diet ((FDA, 21CFR170.235). Exposure data also includes an estimate of dietary exposure to other products generated from the substance, such as breakdown products (FDA, 21CFR170.235). For example, dietary exposure data related to pennycress seed meal might include exposure to the glucosinolate sinigrin as well as the breakdown product isothiocyanate. Exposure data also includes potential contaminants ingested as a result of consuming a substance (FDA, 21CFR170.235). For example, notifiers should include potential exposure to pesticides or herbicides resulting from consumption of an agricultural product. Finally, notifiers must include sources of consumption data and assumptions used to estimate dietary exposure.

GRAS status generally requires evidence from costly feeding studies. Pennycress lacks extensive feeding studies since food grade varieties are not widely available. As production of pennycress varieties with food grade oil increases, it will be important to pursue feeding studies in support of GRAS approval. The cost of such studies might be shared among a variety of public and private stakeholders. Achieving GRAS status for both oil and meal will be essential to unlock the wider production of pennycress.

Table 2.4 - GRAS requirements summary

GRAS Element	Description
Information on notifier	Party requesting GRAS notification
Intended use 21 CFR 170.230	Product types intended to contain the ingredient and role ingredient will play within the product.
Identity 21 CFR 170.230	Characterization of raw material.
Method of manufacture 21 CFR 170.230	Processes and methods used to produce ingredient.
Specifications	Chemical characterization of ingredient.
Dietary exposure 21 CFR 170.235	Levels that will be consumed within a product by the public
Self-limiting levels of use 21 CFR 170.240	Level at which the notified substance renders food unpalatable

2.9.6 Canola Analogy – Production, Product Potential, Consumption, and Supply Chain

As described above, pennycress is closely related to canola, and canola faced many of the same barriers as it was developed from inedible rapeseed, including high levels of erucic acid and glucosinolates. Figure 2.5 provides a timeline that compares the development of canola with pennycress. Recent genetic advances facilitated the identification of a canola-like pennycress oil profile (Chopra et al., 2020). Since pennycress oil and meal might suit similar food applications, an understanding of the canola supply chain along with consumption, production, and historical factors has the potential to provide orientation for a potential pennycress market.

In the early 1900s rapeseed oil was largely used for industrial purposes (e.g. lubricants). Rapeseed was primarily grown in and exported from Europe and Asia during this timeframe (Baranyk & Fabry, 1999). The World Wars in the early-mid 1900s created a

period of unrest in global trade alliances, which resulted in a shortage of rapeseed in North America. Canada began producing rapeseed to satisfy the demand created by the war and soon became a global power in rapeseed production and consumption (Canola Council of Canada, 2020a). In the 1950s, the United States Food and Drug Administration (FDA) banned rapeseed consumption due in part to the high content of erucic acid (*US Canola Association: Seed, Oil, and Meal*, 2020). Notably, the seed also contains high levels of glucosinolates, which limit its utility as a food and feed source. Canadian plant breeders soon developed so-called “00” (double zero- nearly devoid of erucic acid and glucosinolates) rapeseed lines in the 1970s. The 00-rapeseed eventually underwent a name change to canola, meaning “Canadian oil low acid,” (Canola Council of Canada, 2020a). Canola oil attained the Generally Recognized As Safe (GRAS) status from the FDA in 1985. Now, canola oil is the third-most produced vegetable oil in the world, behind palm oil and soybean oil, with a robust supply chain concentrated in Canada (Prentice, 2018).

The canola supply chain produces three different products: whole seeds, oil, and meal. Whole seeds are handled through bulk-commodity mechanisms (Prentice, 2018). In short, seed is transported from a farm to a grain elevator. It is then shipped, usually by railcar or truck, to a larger grain aggregator, processing company, or an export center. Once the seed reaches a processor, it is crushed for oil and meal. A typical Canadian canola crushing facility crushes 3,000 tons per day (Prentice, 2018).

Oil and meal processing include seven steps: seed cleaning, pre-conditioning and flaking, cooking, pressing the flake, solvent extraction, de-solventizing and toasting the meal, and further oil processing (Canola Council of Canada, 2020b). First, seed is cleaned to remove unwanted debris – e.g. stems, weed seeds, damaged seeds, etc. Then, the seed is often heated to prevent shattering. The heated seed is then flaked. This process slightly ruptures the seed coat, which improves pressing efficiency. The flaked seed is then cooked at temperatures ranging 80-105 C. The cooking process further ruptures the seed cells and reduces oil viscosity, which improves pressing efficiency. Cooking also deactivates myrosinase enzymes and prevents breakdown of glucosinolates still present in the seed. The cooked seeds flake then enter a screw press, which extracts 50-60% of the oil. The by-product of this process, press-cake, contains up to 20% oil. The press-cake undergoes a solvent extraction to remove the remaining oil. Here, the press-cake enters an extraction apparatus that continuously floods with hexane, which separates the meal and oil. After a series of solvent washes, the remaining meal usually contains less than 1% oil. The meal is then de-solventized, toasted, dried, and milled to a uniform particle size. If the meal is entering the animal food market, it is often pelletized. The crude oil extracted from the seed and meal is further refined to remove phospholipids, free fatty acids, meal particles, pigments, and gums. Then, oil is further filtered and deodorized to remove unattractive colors and unpleasant odors. Refined oil is then packaged or further processed into other products, like margarine. Refined oil is sold as cooking oil and is transported by rail car or truck to food companies or retail stores. Similarly, meal is transported by rail car or truck to animal feed companies.

Canola is the second-most consumed vegetable oil in the United States, and most of the US canola supply arrives from Canada. According to the Canola Council of Canada, the US imported 490 thousand tons of seed, 1.8 million tons of oil, and 3.4 million tons of meal in 2019, valued at \$3.5 billion (*Canola Council of Canada - Markets and States: United States*, 2020). Canadian canola imports generate \$6.4 billion of economic activity in the US and provide 15,600 US jobs (Canola council of Canada, 2019). In 2019, the United States produced two million acres of canola. Most canola production occurs in North Dakota, which grows 1.8 million acres (USDA-NASS, 2020c). The United States produced 3.4 billion pounds of canola in 2019 with an average yield of 1,661 pounds per acre (USDA-ERS, 2020). Farmers received \$0.14 per pound on average compared to the ten-year average of \$0.18 per pound. Canola crushing outpaced production by four hundred million pounds with 3.8 billion pounds crushed in 2019. The US produced 1.6 billion pounds of oil and used 5.4 billion pounds of oil, including 1.2 billion pounds for biodiesel and 4.2 billion pounds for food use. The average oil price totaled \$0.37 per pound compared to the ten-year average of \$0.43 per pound. Meal production totaled 1 million tons while consumption outpaced production at 4.8 million tons. The average 2019 meal price totaled \$265.00 per ton compared to the ten-year average of \$294.50. Taken together, these data indicate the US consumes far more canola than it produces.

Canola oil is the second-most consumed edible oil in the US, trailing soybean oil. US consumption of canola oil totaled 4.2 billion pounds in 2019 compared to 22.9 billion pounds of soybean oil (USDA-ERS, 2020). Edible oil consumption has increased rapidly

over the last 30 years. In 1991, US canola consumption totaled only 800 million pounds compared to 4.2 billion pounds in 2019.

Canola meal is primarily fed to animals. It is the second most produced and consumed protein ingredient in animal foods, following soybean meal. For instance, US consumption of canola meal totaled 4.8 million tons in 2019 compared to 36.8 million tons of soybean meal (USDA-ERS, 2020). Canola meal contains 40-44% crude protein. In contrast, soybean meal contains 49% crude protein. A growing body of research aims to understand the utility of canola protein in human foods. Canola contains the proteins cruciferin and napin. Canola protein exhibits varying degrees of functionality depending on processing techniques and relative concentrations of cruciferin and napin (L. Campbell et al., 2016). Proposed food categories for canola proteins include bakery products, beverages, egg substitutes, and processed meats (Cambell et al., 2016). The Protein Digestibility Corrected Amino Acid Score (PDCAAS), a crucial measure of protein quality, ranges from 0.61 to 0.86 for canola, similar to soy protein. In contrast, the PDCAAS scores for soy, egg, and milk proteins are 0.86, 0.94, and 0.95, respectively. Multiple canola protein products received GRAS status over the last decade, indicating that these proteins are safe for consumption (FDA, 2010, 2011, 2016a).

There are potential drawbacks to using canola proteins in human foods, including flavor, allergenicity, and stigma around Genetically Modified Organisms. Canola meal harbors a bitter flavor due to the presence of phenolic compounds, glucosinolates and other phytochemicals (L. Campbell et al., 2016; Mailer et al., 2008). These off-flavors limit the

utility of canola meal in a wide range of food applications. Reducing these compounds has the potential to improve flavor and expand the range of products that might include canola meal. Canola protein may be allergenic. The seed proteins napin and cruciferin have been identified as allergenic proteins in yellow mustard, a close relative to canola (L. Campbell et al., 2016). Mustard is recognized as an allergen in Canada and the European Union, and regulatory officials recommend labeling canola protein as an allergen in these countries (*Mustard*, 2020). This potentially limits market value for canola protein and presents a possible risk to allergic consumers. Finally, the stigma surrounding GMOs may hinder the wide consumption of canola protein. According to the Non-GMO project, genetically modified canola varieties producing higher levels of oleic acid became available in the 1990s (Non-GMO Project, 2019, p.). Some consumers are reluctant to purchase products containing GMOs. Notably, pennycress varieties with food grade qualities have been developed with non-GMO breeding techniques. This may position pennycress with an advantage over canola.

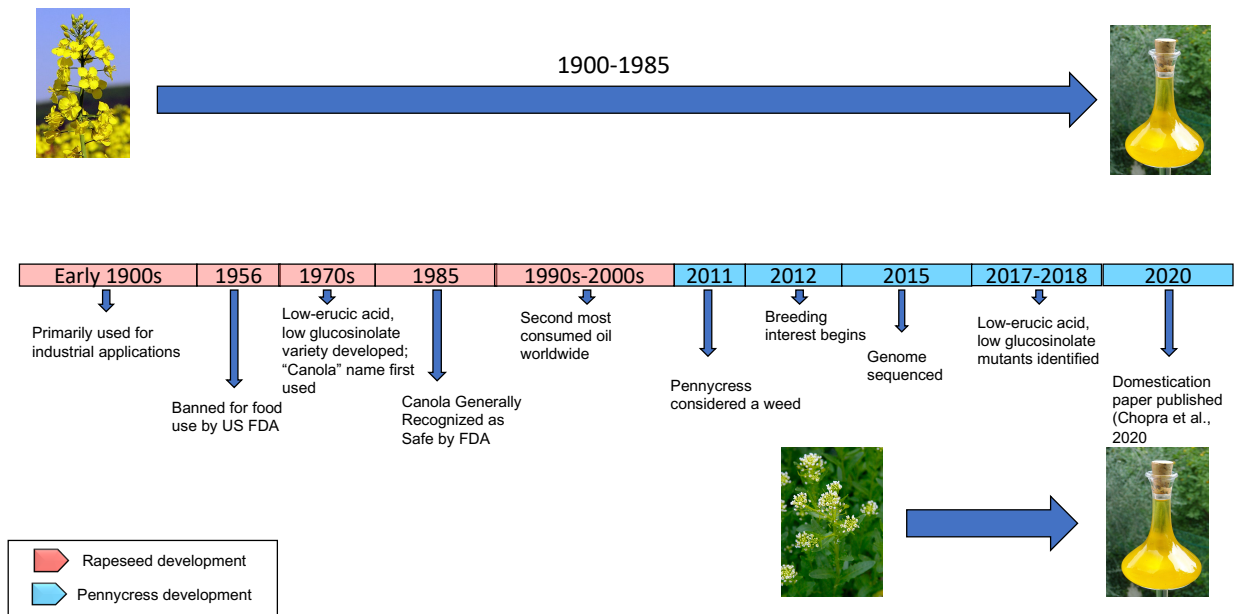


Figure 2.5 - Development timeline for canola and pennycress

2.10 Relevance of our work

Agriculture, food, and nutrition professionals are called upon to develop sustainable, nutrition-forward food solutions that preserve the integrity of the environment, contribute to economic prosperity, maintain optimal health, and remain socially acceptable.

Developing these food solutions requires systems-based approaches to catalyze agricultural innovation and food production. Cultivating these abilities requires capacity building in 21st century food systems professionals, emphasizing cross-disciplinary skillsets within a connected and nimble workforce. Rather than operating in disciplinary silos using reductionist approaches of the 20th century, current nutrition professionals must work across disciplinary boundaries to understand key concepts in multiple food systems fields, such as agricultural sciences, economics, and social sciences.

We demonstrate an interdisciplinary approach that addresses technical and adaptive challenges within the Food System Sustainability paradigm. Our approach and project portfolio align with the four dimensions of the nutrition ecology framework, similar to the nutrition sensitive value chain concept: health (access to whole grains in restaurants and pennycress food quality), economics (whole grain supply chain barriers and pennycress production economics), environment (sustainable crop development), and society (discussions on the land grant university role of creating a more sustainable food system, new training approaches to nutrition). This work provides a roadmap for future systems-based nutrition approaches by providing examples of cross-disciplinary research aimed at improving critical issues throughout the food system, which may be broadly characterized as nutrition sensitive value chain approaches. First, we identify key challenges, barriers and opportunities throughout the whole grain supply chain limiting availability of and access to whole grain products in restaurants. Working in the whole grain supply chain provides an example of working in a conventional, established food system. Then, we focus on improving a new food system for the sustainable crop pennycress. First, we collaborate with plant geneticists to identify genes underlying the accumulation of anti-nutritional glucosinolates. Reducing glucosinolates has the potential to improve the palatability of pennycress seed meal, the byproduct of the oil crushing process. Improving seed meal palatability and reducing antinutritive effects of the meal has the potential to valorize the pennycress supply chain. Then, we collaborate with economists to model the on-farm production economics of pennycress. Understanding pennycress production economics is essential and has the potential to help prioritize

future development projects (e.g. breakeven yield sets a target for future breeding efforts). Then we discuss the role of the land grant university in facilitating the development of sustainable, nutrition-sensitive food systems. Universities sit at the intersection of many food systems actors and drivers, and enhanced coordination and collaboration of these stakeholders has the potential to improve health and environmental food systems outcomes. Then, we discuss interdisciplinary training in the context of food systems improvement. Training the next generation of food systems professionals in interdisciplinary, food-systems concepts can improve efficacy and facilitation of future systems approaches. We recommend a three-step process for initiating future systems approaches in nutrition. These steps include systems mapping, stakeholder engagement, and transformational action. Finally, we discuss broader implications and future applications of our interdisciplinary approach in the context of sustainable, healthful food systems change.

Chapter 3: Challenges and opportunities associated with whole grains use in Twin cities restaurants: A food systems perspective

Abstract:

Increasing whole grain (WG) availability in restaurants allows consumers to make healthier choices with minimal effort while improving adherence to the Dietary Guidelines. To understand challenges associated with increasing WG availability in Twin Cities (Minneapolis & St. Paul, Minnesota, USA) restaurants, interviews and focus groups were conducted with 24 local, national, and international food system members. This report identifies food system barriers, including policy, business, and societal pressures, that limit availability of WG based foods in restaurants. Insight provided by this study allows consortium members from various sectors and disciplines to work on a precompetitive basis to include more WG in Twin Cities restaurants.

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3.1 Introduction

Food environments influence diet and health. These environments are the human-built and social environments that include physical, social, economic, cultural, and political factors that impact accessibility, availability, and adequacy of food within a community or region (Rideout et al., 2015). For example, consumer food environments are characterized by the availability, variety, price, and quality of foods as well as promotional signs and prominence of healthful versus non-healthful food options (Rideout et al., 2015). Restaurants provide one example of a consumer food environment that has a major influence on diet and health.

As consumers continue to rely on restaurants for food, their intake becomes dependent on available menu options. Restaurant meals have been shown to be generally less healthful as these meals provide more calories, total and saturated fat, sodium, and cholesterol per meal than a typical meal prepared at home (Hearst et al., 2013; Scourboutakos & L'Abbé, 2012). Conversely, meals away from home (AFH) can be low in several key nutrients, including fiber, calcium, and iron (Lendway et al., 2014). Research indicates that AFH eating is associated with increased biomarkers of chronic disease (Kant et al., 2015). As the reliance on AFH eating increases, it becomes clear that restaurants must play a role in offering and promoting healthier meal options.

Given the current consumer food environment, actions to improve the nutritional quality of menu options in restaurants remain prudent. Substituting WG's for refined grain products can increase the nutritive value of foods without drastically changing

acceptability and organoleptic qualities of meals (Rosen et al., 2008). The most common grains in the American diet include wheat, oats, rice, maize, and rye. These grains in their whole form contain an abundance of nutrients, including vitamin E, magnesium, iron, fiber, several B vitamins, and other beneficial phytochemicals (Y. Zhu & Sang, 2017). Diets containing WG's are inversely related with obesity, insulin resistance, diabetes, and inflammation (Lutsey et al., 2007). Whole grain consumption is also associated with reduced risk of coronary artery disease, cardiovascular disease, and total mortality (T. Huang et al., 2015). For these reasons, the 2010 and 2015 Dietary Guidelines for Americans recommend that at least half of all grain servings should be WG (US Department of Agriculture and US Department of Health and Human Services, 2010, 2015). More specifically, the Guidelines recommend that all adults and children over 9 years consume at least 3 servings of WG's per day. However, data from the United States Department of Agriculture (USDA) showed that Americans only consumed an average of 0.90 servings of WG's per day in 2013-2014 (Bowman et al., 2017). Results from the 2009-2010 National Health and Nutrition Examination Survey (NHANES) indicated that only 2.9% of children and adolescents (2-18 years old) and 7.7% of adults (over 18 years old) met the recommended intake of WGs (Reicks et al., 2014). Increasing the availability of WG based foods in restaurants could allow consumers to make healthier choices with minimal knowledge and effort while also improving their adherence to the Dietary Guidelines.

Currently, barriers exist within the food system and food environment that prevent the incorporation and increased availability of WG based foods in restaurants. These barriers

include limited knowledge regarding WG foods, undesirable taste or texture qualities, increased preparation time, higher cost, and low availability in foodservice settings (Jacques et al., 2013; Rosen et al., 2011). Traditionally, the responsibility has fallen on consumers to manage their health by actively seeking nutritious foods when making dietary choices. However, several studies indicate that the current consumer food environment obstructs, rather than supports, healthy eating habits (Harvard School of Public Health, 2015). For this reason, our study focused on factors across the supply chain that limit whole grain use in Twin Cities (Minneapolis and St. Paul) restaurants beyond consumer and restaurateur preferences. A systems perspective demonstrates that, while the ultimate decision of whether to consume WG's lies with the consumer, the circumstances which most greatly influence that decision are pre-determined long before the consumer reaches the restaurant. The findings of this study may elucidate ways to utilize the supply chain and restaurant settings to make healthier, WG menu options a more effortless and instinctive choice for consumers when eating out.

3.2 Methods

The Institutional Review Board (IRB) at the University of Minnesota approved this study. Individual interviews (n=24) were conducted during the summer of 2013 with a convenience sample of local, national, and international food system members that contribute to the Twin Cities restaurants. Individual interview participants included culinary (n=4), baking (n=2), milling (n=5), food supply chain (n=7), public health (n=2), and academic professionals (n=4). Participants were selected from throughout the WG food system so researchers could understand a broad a range of perspectives. Most interviews were 45 minutes to one hour in duration. Interviews were generally informal

and allowed for deviation from the original interview questions. This method was chosen to understand broad themes related to different sectors and disciplines involved in the WG food system. Sessions were audiotaped and transcribed verbatim. Two investigators then independently coded transcripts to generate themes and ascribe them to the appropriate tiers of an Adapted Social-Ecological Model (ASEM) (Richard et al., 2011).

During interviews, participants were asked questions related to the following: (1) The participant's role in influencing WG consumption; (2) The influence of the grain supply chain on WG consumption in restaurants; (3) The influence of public health initiatives on WG consumption in restaurants; (4) The specific barriers preventing WG availability and consumption in restaurants; (5) Potential strategies for increasing WG availability and consumption in restaurants; and (6) Intra-sector and inter-sector collaboration between the participant and other professionals.

3.2.1 The adapted social-ecological model

The Adapted Social-Ecological Model (ASEM) is a five-tier adaptation of what is traditionally a four-tiered model (Richard et al., 2011). The Social-Ecological Model includes the following tiers: Individual, Interpersonal, Built Environment, and Policy/Society. In the ASEM, the "Built Environment" tier is subdivided into "Restaurants" and "Supply Chain." The ASEM more accurately represents the relationship between the two subcategories and the end consumer. Within the context of AFH eating, restaurants have direct influence on consumer choices, while the supply chain typically has an indirect influence through the restaurants (menu options). The ASEM demonstrates how consequences of the supply chain/restaurant relationship,

which would be lost in a traditional model, have tremendous influence on consumer behavior (Richard et al., 2011). Moreover, the adoption of WG presents issues in restaurants, which would not apply upstream in the supply chain, and vice-versa. This model preserves the unique needs of each tier, while demonstrating their interconnectedness and cascading effects on the end consumer.

3.3 Results

Several themes were identified from participant responses. These themes have been categorized according to the applicable tiers of the ASEM. The themes and challenges in each tier of the ASEM will be addressed in ascending order, beginning at the individual level, and culminating with Policy/Societal influence. General themes related to participant beliefs about opportunities are also presented for each ASEM tier.

3.3.1 Individual

Interview evaluations revealed four major barriers to WG consumption in restaurants on the individual level. These themes included: (1) health stigma, (2) sensory characteristics, (3) cost, and (4) availability, as a majority of participant responses focused on the individual level.

3.3.1.1 Health stigma

Eleven respondents indicated the common perception of WG as a “health food” hinders consumption in restaurants. Although most participants acknowledged that some people seek WG when eating out, the majority is discouraged due to its reputation as “health food.” “WG can easily be stigmatized as ‘healthy’ and healthy is death for consumer demand in many circles (Academic).” This mentality was reflected well by one quote

from a public health professional who commented, “Some people go specifically to indulge themselves in restaurants, and don’t want to be reminded that they’re eating things that aren’t necessarily the best for them...people want to indulge, and they see WG not as an indulgence, but as something that isn’t as good.” An academic expressed a similar sentiment, saying, “I think to a certain extent, sometimes people get saturated with the health message. And so they may have a demand, but they don’t want to be told all the time that they have to be healthy.”

3.3.1.2 Sensory characteristics

Seven respondents indicated that unpleasant taste or textural characteristics in WG foods are a significant deterrent to their appeal with consumers. Whole grain products contain compounds that are not present in their refined counterparts. These compounds alter the taste of the final product, as well as its texture and color. Whole grain products have been described as “bitterer”, “denser”, “heavier” and “earthier” tasting than the refined version of the same product. Yet, there was little interest and/or motivation by respondents to want to overcome challenges and seek opportunities to develop and deliver WG foods that consumers desire and want to eat.

3.3.1.3 Cost

Six respondents indicated that the increased costs associated with WG foods prevent their consumption in restaurants. Whole grain products are often considered premium items due to the relatively low demand for these products and the difficulty of procuring them on a large scale. Until the demand for WG’s reaches a critical mass, the supply will constantly be insufficient to meet the needs of those who require the products in greater

volumes, such as restaurants. Whole grain products have a shorter shelf life than refined grain products, which limits the time it can spend in distribution centers or warehouses. Thus, most distributors only acquire the quantity necessary to meet current customer demand. This demand is often low because restaurants must pay a premium to acquire many WG products in the quantities necessary to serve their clientele. This cost is invariably passed on to the consumer who may or may not even be willing to try the dish. Restaurants will not sacrifice storage space, equipment, and profits for a product that may sit on a shelf until it expires, or worse, may alienate their customers. Rather than take this risk, a restaurateur may opt for the cheaper refined grain option that is more likely accepted by the majority.

3.3.1.4 Availability of added value grain-based foods

Several respondents indicated that low availability of quality WG prevented widespread usage in restaurants. Frequently voiced opportunities included increasing the availability of popular or frequently consumed foods in restaurants that can be made with WGs that are healthier, cost-effective, and desirable. These WG foods would be readily accessible to restaurant clientele and easy for chefs and food service personnel to purchase, prepare and serve in an efficient and economical manner. Children were also mentioned as a primary demographic targeted for greater exposure to WG foods.

3.3.2 Interpersonal

Few people communicated or initiated efforts related to WG's within the supply chain and food environment. Millers and bakers commented that a majority of their work revolves around communication related to refined grain flour and their respective baked

products. Overall, participant responses about communication and collaboration were less frequently voiced relative to the use of WG's. From a chef's point of view, "The only collaboration I have about WG's is people want me to use their ingredients, so I can sell it to the customer." This may indicate a lack of support in restaurants to help chefs succeed using WG ingredients and WG foods that restaurant clientele will eat. On the other hand, it was voiced "People [Patrons] don't really have the venue to make their preferences known in restaurants." Participants suggested opportunities may exist to better understand the value and meaning of WG's relative to supply chain players, restaurant owners, managers, and clientele. A deeper examination of the potential roles, functions and activities of these players might help gauge more appropriate use of WG foods in restaurants.

3.3.3 Restaurants

Participants reported that chefs and the general public are often intimidated by new experiences like preparing, serving, and eating WG. Other participants indicated restaurants have no immediate incentive to use WG, thus "Higher quality ingredients do not necessarily equal higher quality products." These ingredients can also be more expensive with no guaranteed reward. As a means to counter this lack of skill and hands-on experience, most participants suggested chefs, cooks and restaurant staff need more training. Currently, there are few to no dollars spent teaching people how to work with WG. As a result, chefs, cooks, and restaurant staff lack foundational skills in purchasing, preparing, storing, and serving these foods. Ultimately, the limited foundation through the food industry, consumers, chefs, and others inhibits whole grain adoption. Thus, a majority of participants suggested considerable benefit might result in training restaurant

chefs, kitchen personnel, and serving staff in purchasing, storing, preparing and marketing popular foods made with WG.

3.3.3.1 Supply chain

Study participants with primary roles within the supply chain stated, "...the supply chain is engineered to produce refined products in large quantities, unlike WG." As mentioned by a wheat breeder, "wheat is bred for optimally refined grain foods, while flour quality is based on refined grain product quality and yield, not for WG foods." From a miller's perspective, there are unique processing needs for WG. In some instances, WG milling requires capital investments in new equipment, such as additional storage silos and milling technology. It was stated strongly that academic interests and research priorities are seldom aligned with industry or consumer priorities. Thus, one food scientist indicated a tremendous need for cross-sector and cross-disciplinary training whereby faculty spend time in industry while industry scientists collaborate more intimately with universities. The intent was to increase understanding and appreciation for collaborative efforts around healthier grain-based foods. Systematically mapping barriers and opportunities in the development, delivery, and consumption of WG foods would allow for a more comprehensive look at the gaps in the supply chain, food environment, and community relative to WG use in restaurants. Additional insight from this effort could provide a means for prioritizing precompetitive and relevant research and education focus.

3.3.4 Policy / societal

From a policy and societal perspective white bread is ingrained in American culture. Most participants commented they engage in a greater frequency of refined grain activities compared to WG's across the sectors and disciplines within the food system. Overall, most activities were reactive (e.g. creating press releases against low carb, gluten free, etc.) rather than promoting the health attributes of grains through the inclusion of WG and fiber in frequently consumed foods.

WG's are not nearly as available through distributors in comparison to refined grains. For example, McDonald's tried to offer a whole wheat bun once, but stopped because customers preferred a white bun. From the perspective of general societal understanding of WG, one industry representative said, "whole grain definitions are too ambiguous and confusing. Consumers don't understand what WG means and neither do the chefs cooking in restaurants."

Based on participant input, opportunities may exist through a unified inter-sector collaboration by developing and implementing a comprehensive public health campaign to build positive messaging around WG foods. Focus would be encouraged through education about WG in harmony with greater availability of WG foods in the marketplace.

3.4 Discussion

This study examined current roles and perceptions of supply chain members, along with challenges and opportunities that each member experienced in bringing WG's from field to fork. Objectives were based on the 2010 Dietary Guidelines which asked for new food

introductions and reformulation of prepared foods that deliver good taste and convenience while meeting dietary recommendations and cost constraints (US Department of Agriculture and US Department of Health and Human Services, 2010, 2015).

The food environment remains mostly devoid of viable WG choices for most Americans, as consumers fail to meet the Dietary Guidelines recommendations which state “at least half of all grain servings should be WGs” (US Department of Agriculture and US Department of Health and Human Services, 2010, 2015). Study participants consistently echoed an overarching theme, that there is little translation of agricultural food policy and dietary guidance into easy access to WG foods, which allows consumers to eat more. From a historical perspective, the grain industry is designed to proliferate the continued milling and sourcing of refined grain ingredients for baking and food manufacturing. In contrast, the WG infrastructure lacks the tools, approaches, methods, foundational knowledge, and standard business practices necessary to carry healthful WG foods from field to consumer. The refined grain system is generally more efficient and cost effective to source, process, market and profit by selling refined grain foods that are practical, affordable and desirable for consumers. Based on this sample of participants, there appears to be a lack of shared value and meaning around WG food-related collaboration and communication strategies.

Barriers to using WG’s occur throughout the supply chain from growing the grain (i.e. red wheat bred for refined grain use, lack of incentives to grow white wheat; pre-

sprouting of white wheat places profits at risk), milling of grain into WG ingredients (different particle sizes result in varying functionalities, more enzymatic activity, lipids, and antioxidants which effect end-use and storage properties), baking and manufacturing into foods (lack of standard approaches and methods for WG product development; shorter shelf-life and storage), distributing and purchasing (lack of WG volume, distribution channels and cost constraints), preparing and serving in a restaurant setting (chef, production and serving staff are unfamiliar with WG ingredient storage, WG food preparation, and service) and consumer needs, wants and desires (lack of availability and accessibility of tasty added value WG foods). Until the necessary tools, approaches and WG infrastructure are established and leveraged in an intentional, synchronous and consistent manner throughout the grains supply chain and food environment, there is little motivation for players in the supply chain to support, encourage or use WG ingredients.

Restaurants are encouraged to gradually shift to incorporate more WG menu options so that AFH consumers can come closer to meeting WG recommendations. Perhaps a gradual increase in the availability of WG foods in schools and in restaurants is one approach to increasing consumption. For example, “sneaking in” better-for-you WG foods in school cafeterias and in restaurants without identifying it on the menu has the potential to be successful. Small recipe substitutions often go unnoticed, and some food and nutrition service directors already do this in hospital cafeterias. This practice is known as “stealth health”, and it is a way to improve the nutritional quality of foods without customers even noticing (Food Service Director, 2012).

An example of increasing WG's in the food supply is to reformulate a popular food product such as pizza. Previous research has shown that modifications of pizza crust to include WG flour have been made without affecting acceptance among school children (Chan et al., 2008). This type of pizza is currently available for school meals but has limited availability in other food service and retail markets (Schwan's, 2020). Pizza can also be reformulated to be WG rich, lower sodium, and lower fat. Since pizza is made up of different food groups and is widely consumed and accepted; it has the potential to be a healthier staple that targets acceptable levels of WG's, vegetables, and dairy with lower fat and sodium. Pizza is just one way that foods can be modified to more closely meet Dietary Guidelines recommendations while satisfying consumer expectations for cost, taste and convenience (Jacques et al., 2013).

Efforts to gradually introduce more WG's into the marketplace provide new, cutting edge opportunities for collaboration throughout the grain community. Cross-disciplinary and cross-sector collaborations will help facilitate progress toward novel approaches to research, discovery, development, and delivery of WG based foods while collectively solving major gaps in the WG supply chain and food environment. Priorities and focus might be established on a precompetitive basis among the sectors and disciplines, related to the type of grain and food product, and place of service in the community, such as introducing WG pizza into restaurants (Tritt et al., 2015). To accomplish this system-wide goal, it requires synchronizing individual (players) and organizational contributions around a grand challenge resulting in collective impact. Adopting a culture of systems thinking while reconfiguring tools, approaches, and food design to emphasize shared

value for profit and health can allow the grains community to overcome limitations in the current paradigm.

Scientists must continue to conduct research on WG and health to solidify the knowledge base, while industry has the unique opportunity to reformulate products to make them healthier (McKeown et al., 2013). Examples of tools, techniques, and methods that might be developed include analytical methods for determining particle size, stability and rancidity in WG ingredients and foods. National and global institutions such as the International Association of Cereal Science and Technology (ICC) can play a major role in allowing these methods and techniques to be available to cereal scientists, product developers and bakers. Standard methods for using WG ingredients in grain-based foods such as bread, tortillas, pasta, cereals, and other baked products provide a precompetitive base for all bakers and manufacturers to develop and deliver better WG foods.

3.5 Study limitations and strengths

We acknowledge the limitations and strengths of this research. One of the limitations of this study was that a convenience sample was used to assess the barriers that exist within the food system and food environment to incorporate WG foods in restaurants. The sample size (n=24) was also small which prevents the generalization of results on a large scale. Finally, one of the other major drawbacks to a convenience sample is the opportunity for bias to cloud the results. In terms of strengths, even though the sample was small, the individuals surveyed represented different sectors of the food system (i.e. academics, non-profit organizations, public health, government, and industry). Having perspective from these diverse disciplines is critical for developing practical solutions to

deliver WG's and WG foods into the food supply. Although the methods are not fully objective the intent of the paper was to show the 'big-picture' interrelationships amongst the roles, functions, and activities that take place throughout the whole grain food system.

3.6 Conclusion

Strategies to increase WG intake in the US diet should target action at each level within the food system and food environment, such as in restaurants. Restaurateurs are apprehensive in the purchase, preparation, and service of WG foods. This may be attributed to unfamiliarity, insufficient demand, shorter shelf life, and potential loss of profit. A WG/health stigma interferes with restaurant clientele selection and consumption of WG foods. Consumers are looking for WG foods that are affordable, and thus, readily available. Although incorporating WG's into restaurants may not solve all these health problems, it is a step in the right direction, especially for young children who can establish healthy eating patterns at an early life stage. Based on this assessment, we will continue to work with a consortium of members from various sectors and disciplines to identify challenges and opportunities for the inclusion of more WG's in the Twin Cities (Minneapolis and St. Paul). The question remains: How do we create a supply chain and food environment that supports a culture of developing, delivering, and increasing consumption of WG foods where interdependent stakeholders can perform their individual roles and effectively collaborate with each other? In part, added value for grain-based foods lies in the strategic use of WG flour to carefully achieve refined to WG flour ratios in most foods that are practical, healthier, affordable, and desirable.

3.7 Relevance to the nutrition ecology framework and the food system

This project primarily aligned with the health and economic dimensions of the nutrition ecology framework. The health dimension encompasses concepts like food security, access, and food quality. Whole grain foods are generally considered healthful foods with high nutritional value. Activities within this project aimed to understand key supply chain barriers to whole grain availability and access in restaurants. System approaches to overcoming these barriers could result in wider availability and access to whole grains, which could ultimately improve food and nutrition security. The economic dimension of the nutrition ecology approach encompasses market, industry, and other economic considerations. Activities throughout this project aimed to understand industry perception and market factors (e.g. costs) that influence whole grain availability in restaurants. The stakeholder perceptions and considerations identified in this study can help inform system-wide strategies to increase availability of whole grain products in food service settings, which could ultimately improve food and nutrition security.

Chapter 4: Identification of reduced glucosinolate mutants in field pennycress (*Thlaspi arvense* L.) by large-scale screening of mutant populations through forward and reverse genetics

Abstract

Field pennycress (*Thlaspi arvense* L.) is being developed as a new winter annual oilseed cover crop that provides ecosystem services when grown between traditional summer crops. Previously, it was shown that using related *Arabidopsis* as a guide, it was possible to identify genes controlling various plant development processes and crucial domestication traits in pennycress. In this study, we used both a forward and reverse genetics approach to identify candidate mutations for genes responsible for a reduced glucosinolate phenotype. First, we used brassica NIRS to screen EMS populations for reductions in seed glucosinolates. Then, we used a pennycress gene mutation index to identify EMS induced mutant alleles in many of the well-studied genes in *Arabidopsis*. We selected *myc3* and *cyp83a1* mutant alleles to understand the effect of the mutations on the genes involved in glucosinolate biosynthesis based on *Arabidopsis*. Overall, these results indicate the success of using an EMS mutagenesis approach to identify candidate genes for pennycress glucosinolate biosynthesis. This strategy will be effective for advancing genetics in pennycress and many other Brassicas. Finally, this approach also provides an opportunity to discover new information on glucosinolate and other metabolite pathways.

An abstract based on this work, entitled “Identification and Characterization of Genes Involved in Field Pennycress (*Thlaspi arvense* L.) Glucosinolate Production,” appeared in the journal *Current Developments in Nutrition*, and it was presented to the American Society of Nutrition. Ratan Chopra, Len Marquart, Nickolas Anderson, Joe Lyons, Tim Ulmasov, Donald Wyse, and M. David Marks contributed to this work.

4.1 Introduction

The grand challenges throughout the global food system, including the triple burden of malnutrition and environmental degradation (M. Clark et al., 2018), necessitate the development of new crops that contribute to economic, environmental, and societal vitality (N.R. Jordan et al., 2016). Global leaders, including the Food and Agriculture Organization of the United Nations, advocate for cross-cutting agricultural innovation that contributes to healthful, sustainable food systems (Barbier & Burgess, 2017). New crops that contribute to biodynamic, regenerative agriculture are undergoing development for food and feed use (Tyl et al., 2020). For example, field pennycress (*Thlaspi arvense* L., pennycress herein) is being transformed into a new oilseed crop that contributes to sustainable agriculture with potential for food and feed applications (Chopra et al., 2020). This transformation is being guided through the use of information derived from research on the closely related *Arabidopsis thaliana* (Chopra et al., 2018), a simple manipulatable diploid genome (K. M. Dorn et al., 2015), development of editing tools (McGinn et al., 2018) and techniques (Chopra et al., 2018, 2019), and a road map for crucial traits (Sedbrook et al., 2014).

Pennycress contributes to environmental vitality as a cover crop. Recent efforts aim to develop pennycress to fit within conventional crop rotations - e.g. planted following corn harvest and harvested prior to soybean planting (Phippen & Phippen, 2012). As a cold hardy, winter annual, the crop is well-positioned to withstand the harsh winters of the Upper Midwest, providing continuous living cover on lands left barren in traditional crop rotations (Johnson et al., 2015). During the winter fallow period, pennycress provides

ecosystem services by preventing soil erosion and nitrogen leaching, sequestering key soil nutrients (Weyers et al., 2019), and providing a food source for early spring pollinators (Eberle et al., 2015). These ecosystem services ultimately improve biodiversity and mitigate negative environmental impacts of conventional agriculture.

Beyond ecosystem services and environmental vitality, pennycress has the potential to contribute to the food system through oil, feed, and fiber. Pennycress seeds contain 30-35% oil, 20-27% protein, and 15% fiber (Evangelista et al., 2012). Pennycress seed meal, produced as a byproduct of the oil crushing process, might serve as a protein source for food and feed applications with functional qualities similar to canola or soy protein (Hojilla-Evangelista et al., 2015). However, in order to utilize pennycress seed meal as a food and feed source, pennycress seed glucosinolates must be reduced. Wild pennycress contains glucosinolates at a level of 92.3 $\mu\text{mol/g}$ seed (Chopra et al., 2019), occurring predominantly as the aliphatic glucosinolate sinigrin (Daxenbichler et al., 1991).

Glucosinolates are considered non-nutritive (i.e. anti-nutrients) substances in humans and animals (J. M. Bell, 1993; Harvard, 2020; Mailer et al., 2008). While some studies report that glucosinolates found in cruciferous vegetables are associated with positive health outcomes, including associations with decreased cancer risk (Hayes et al., 2008; Ma, Liu, Sampson, et al., 2018; Ma, Liu, Zong, et al., 2018), animals fed high glucosinolate oilseed meal experience decreased weight gain, decreased thyroid function (e.g. goiter), and other negative health effects (Bourdon & Aumaître, 1990; Landero et al., 2018; Tripathi & Mishra, 2007; L. P. Zhu et al., 2018). Historically, glucosinolate reduction has been a major target for increasing the value of several important oilseeds including

rapeseed, canola, and pennycress (J. M. Bell, 1993; Mejicanos et al., 2016; Sedbrook et al., 2014). While wild pennycress contains glucosinolates at a level of 92.3 $\mu\text{mol/g}$ seed, canola contains glucosinolates at a level of 10-15 $\mu\text{mol/g}$ seed on average, with standards requiring less than 30 $\mu\text{mol/g}$ oil free meal (European Food Safety Authority (EFSA), 2008; NSW Department of Primary Industries, 2014). Reducing glucosinolates in these seeds is imperative to valorize waste streams resulting from oil pressing, which can improve the profitability of the pennycress system.

Glucosinolates are a diverse class of secondary metabolites produced in brassicas, including broccoli, cabbage, cauliflower, brussels sprouts, horseradish, mustard, and oilseed rape (Barba et al., 2016; Fahey et al., 2001). These sulfur-rich compounds contribute to the pungent aromas and sharp, bitter flavors associated with these plants (Wieczorek et al., 2018). Glucosinolates are synthesized throughout the plant and function as defense compounds that protect against herbivores, insects, and other pathogens (del Carmen Martínez-Ballesta et al., 2013; Singh, 2017). Once plant tissue is ruptured by chewing or cutting, myrosinase thioglucosidase enzymes catalyze glucosinolate hydrolysis, leading to the formation of highly reactive breakdown products including thiocyanates, isothiocyanates, indoles, oxazolidine-2 thiones, epithionitrile, and nitriles (Bhat & Vyas, 2019; Fenwick & Heaney, 1983). These breakdown products are responsible for the defensive function of glucosinolates. High-heat processing techniques deactivate myrosinases; however, bacteria in human and animal digestive systems function similarly to the enzymes (Ishida et al., 2014). Therefore, reducing glucosinolates

via genetics and breeding techniques is more effective at minimizing their negative effects.

The structure, biosynthesis, and metabolism of glucosinolates have been extensively studied in several plants throughout the *Brassicaceae* family, including in the model plant *Arabidopsis thaliana* (Figure 4.1) (L. Bell, 2019; Blažević et al., 2020; Glawischnig et al., 2003; Ida E. Sønderby et al., 2010). Glucosinolates are derived from an amino acid, a thioglucose moiety, and a sulfonated oxime. They are classified as indolic, aromatic, or aliphatic depending on their amino acid precursors. Glucosinolate biosynthesis includes three reactions mediated by many different enzymes and regulated by several transcription factors. Figure 4.1 illustrates the glucosinolate biosynthesis pathway. First, an amino acid, such as methionine, the precursor for sinigrin, undergoes a side chain elongation by BCAT3 (Knill et al., 2008), BCAT4 (Schuster et al., 2006), and MAM1 (Kroymann et al., 2001) enzymes. Then the chain-elongated amino acid undergoes a series of oxidation and sulfation reactions by CYP79F1 (S. Chen et al., 2003), CYP83A1 (Naur et al., 2003), UGT74B1 (Douglas Grubb et al., 2004) and SUR1 (Mikkelsen et al., 2004) that generate the core glucosinolate structure. Finally, the core structure undergoes secondary side chain modifications by FMO GS-OX1 (B. G. Hansen et al., 2007), SOT18, AOP1, and AOP2 (Neal et al., 2010). These secondary modifications are responsible for differentiating glucosinolates and contribute to the diversity of the compound. Hundreds of different glucosinolates have been identified, and several different glucosinolates can occur in a single plant. In addition, transcription factors such as MYB28 (Gigolashvili et al., 2007; Ida Elken Sønderby et al., 2007, p. 28), MYB29

(Gigolashvili et al., 2008; Ida Elken Sønderby et al., 2007), MYB76 (Gigolashvili et al., 2008, p. 76), MYC2 (Schweizer et al., 2013), MYC3 (Schweizer et al., 2013), MYC4 (Schweizer et al., 2013), MYB51 (Frerigmann & Gigolashvili, 2014), and MYB118 (Yuanyuan Zhang et al., 2015) form complexes that regulate glucosinolate production and mediate metabolite flux between other secondary metabolite pathways. Therefore, identification of glucosinolate mutants can be difficult as many genes are involved in production, and many loss of function (*lof*) glucosinolate mutations influence plant development and fitness (Douglas Grubb et al., 2004; Hemm et al., 2003; Mikkelsen et al., 2004).

Forward and reverse genetics approaches have led to the identification of many traits in pennycress, and this has been possible due to the simple diploid genome and the one-to-one correspondence of genes with the model plant *Arabidopsis* (Chopra et al., 2018; K. M. Dorn et al., 2015). Building off these approaches, we aimed to identify low-glucosinolate mutants and mutations associated with the low-glucosinolate phenotype. In this study, we used forward- and reverse genetics approaches to identify candidate mutations in genes responsible for a reduced glucosinolate phenotype. The forward genetics approach used brassica NIRS to screen EMS populations for plants with reduced seed glucosinolates. The reverse genetics approach used a pennycress gene mutation index to identify EMS induced mutations in alleles in many of the well-studied genes in *Arabidopsis* (Figure 4.1). We selected mutant alleles from two genes involved in glucosinolate biosynthesis for further validation.

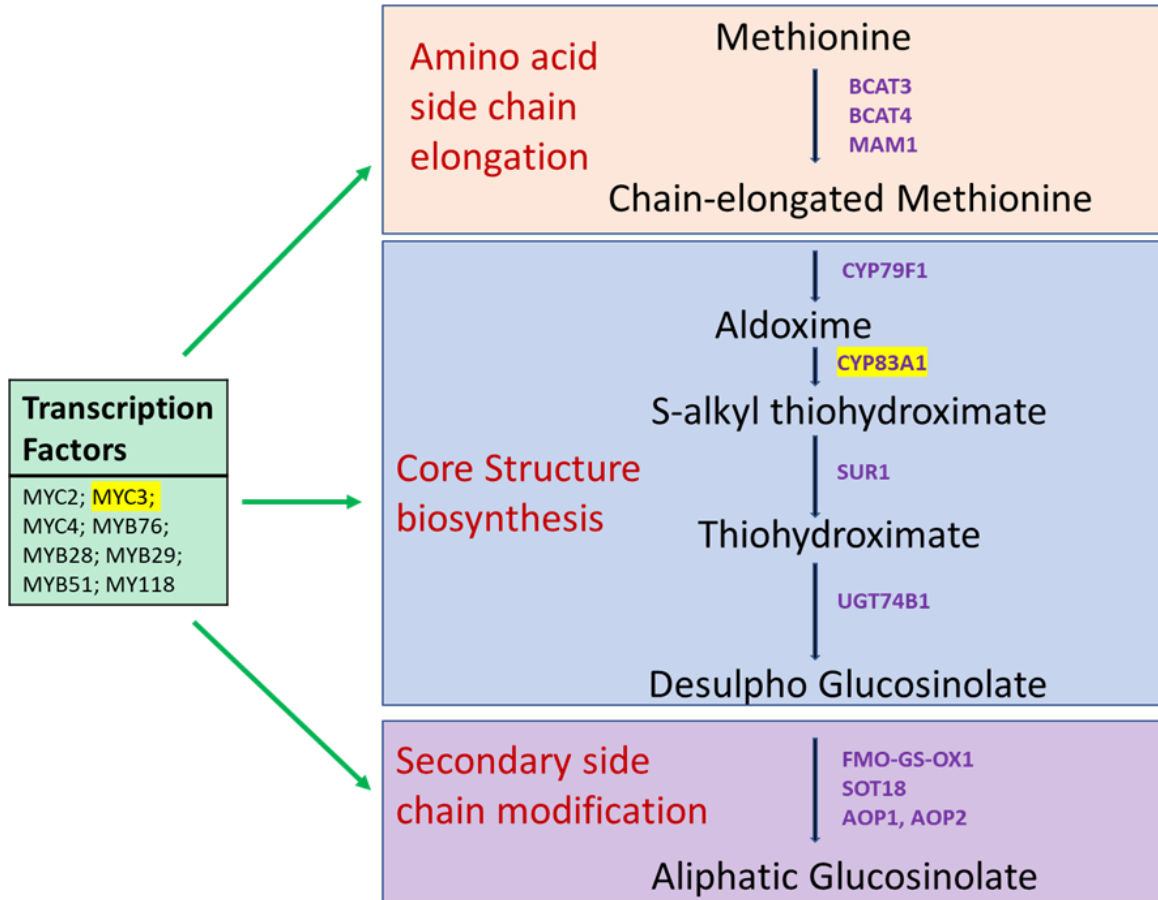


Figure 4.1 - Proposed aliphatic glucosinolate pathway in pennycress based on Arabidopsis. Pennycress orthologs selected for validation in this study are highlighted in yellow.

4.2 Methods

4.2.1 NIRS and UV-based glucosinolate analysis of pennycress seeds

To identify reduced glucosinolate mutants we used the EMS populations generated previously (Chopra *et al.* 2018) followed by the NIRS prediction protocol described in Chopra *et al.* (Chopra *et al.* 2019). Briefly, spectra were collected at room temperature on a DA 7250 NIR spectrometer (Pertten Instruments, Hägersten, Sweden) using the Micro Mirror Module™ from Pertten Instruments with a 950 to 1,650 nm wavelength range and a scan resolution of 5 nm. Brassica calibration equations were used to predict

chemical composition. To determine glucosinolate content of single seeds or validate glucosinolate levels in the candidate mutants identified using NIRS, we used a UV-based method. In brief, glucosinolates were extracted in 80% methanol that keeps the glucosinolate degrading enzyme (myrosinase) inactive and then loaded onto a DEAE Sephadex A-25 mini-column followed by washing and incubation with sulfatase to release desulfoglucosinolates. The resulting desulfosinigrin was collected and quantified by UV absorbance at 230 nm using an internal standard obtained from Sigma (St. Louis, MO). An average of three data points was used for estimating the glucosinolate content in each of the samples, respectively.

4.2.1 Mutant gene index (MGI) for target selection

To identify putative candidates for reduced glucosinolate mutants, we screened for genes in the gene mutant index described by Chopra et al (Chopra *et al.* 2018). Based on the studies in model plant *Arabidopsis* for glucosinolate biosynthesis, we selected genes described in Figure 4.1 and performed searches in the mutation list.

4.2.2 Sequence analysis

Protein sequence alignments were performed using the sequences from *Thalspi arvense* and *Arabidopsis thaliana* for candidate genes. Mutation sites of the candidate genes were changed manually, and alignments were performed using the clustalW program. Protein sequences alignments for all wild-type and mutant genes discussed in this article are provided in Supplementary File 1 (Appendix 1).

4.2.3 DNA extraction and genotyping

DNA was extracted using the Sigma Extract-N-Amp Plant Extraction and Dilution Solution (Sigma-Aldrich, D5688 and E7526) from the seed tissue or leaf tissue, respectively. Allele-specific genotyping was performed on a LightCycler 480 (Roche, Branford, CT) using the protocol described in Chopra et al. (Chopra *et al.* 2018) and primers described in Supplementary Table 2 (Appendix 1) for the *myc3-2* F2 population. To evaluate the effect of the mutation in *cyp83a1-3*, we used single seed genotypes and metabolite analysis for co-segregation analysis described in Chopra et al (2019) for a total of 24 M4 seeds with some modifications for metabolite analysis. In this study, we extracted glucosinolates from single seeds as described above and the remaining material was used for genotyping.

4.2.4 RNA extraction and expression analysis

Leaf tissue was collected from flowering plants and used to extract RNA from wild-type (MN106), *myc3-2* and *cyp83a1-3* mutants. Three replicates for each line were extracted separately using the RNAeasy mini clean up kit (Qiagen, Valencia, CA) and treated with turbo DNase (ThermoFisher #AM2238). To evaluate the expression differences, qRT-PCR primers were designed for actin, *MAMI*, *CYP83A1* and *AOP2-like* (Supplementary Table 2 – Appendix 1). Briefly, qRT-PCR was performed using copy-DNA libraries generated from the total RNA using the Invitrogen cDNA synthesis kit (Invitrogen, Grand Island, NY). The qRT-PCR was performed on cDNA from the four samples with 3 biological and 3 technical replicates using SybrGreen on a LightCycler 480. Expression of mutants and wild-type were normalized using the references genes, and we report the fold change in mutants compared to the wild-type.

4.2.5 Statistical analysis

A student test and a pairwise Tukey test were performed to estimate significance among each class analyzed in this study using JMP 13.2.1 (SAS Institute Inc., Raleigh, NC). Differences in means for each of the fatty acids measured were assessed using means comparisons with a statistical significance at p-value of 0.05 or 0.10, respectively. Chi-squared (χ^2) tests were used to quantify the goodness of fit between expected and observed allelic ratios.

4.3 Results

4.3.1 Forward genetics to identify Low-glucosinolate pennycress mutants

Previously, we validated the use of Brassica NIRS calibrations to identify variation in pennycress seed composition (Chopra et al., 2019). In that report, we found hundreds of samples with reduced levels of glucosinolates, and we selected 112 samples to validate the seed glucosinolate NIRS predictions. The results suggested moderate correlation with wet-lab spectrophotometer analysis. In this study, we included an additional 30 lines, including the wild type (MN106), that showed a range of variation based on NIRS predictions for wet lab validation (Figure 4.2A; Supplementary Table 1- Appendix 1). Combining the previously published data with the new data generated in this study, we found moderate correlation between the wet-lab and NIRS as previously observed (Figure 4.2B). These results suggest that we can continuously use NIRS predictions to select low-glucosinolate mutants from populations or to predict profiles for mutants identified through reverse genetics.

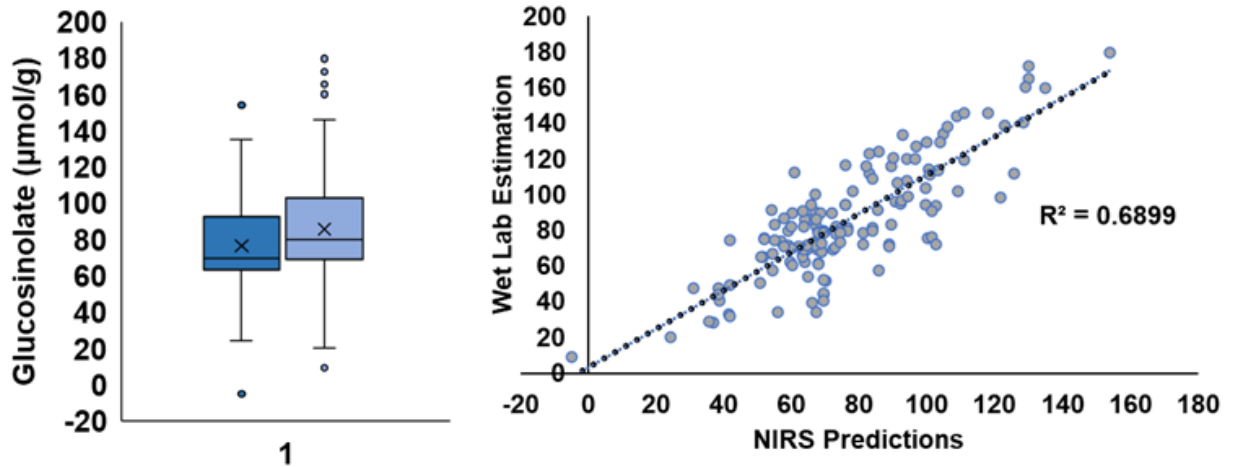


Figure 4.2 - A) Box plot representation of NIRS predictions and wet-lab analysis of pennycress seed glucosinolates, B) Correlation plot of NIRS predictions and wet-lab analysis of pennycress seed glucosinolates

4.3.2 Reverse genetics to identify target genes in pennycress glucosinolate mutants

To identify potential target genes, we searched for pennycress orthologs involved in glucosinolate biosynthesis based on similarity to Arabidopsis, with a focus on genes with single copies. Nine genes showed one-to-one synteny with Arabidopsis. To identify target genes efficiently, we used a pennycress gene mutation index which has partial and complete loss of function alleles in 70% of the pennycress orthologs. In this database, we found 23 lines carrying mutations in predicted glucosinolate orthologs (Table 4.1).

Mutations were present in the transcription factor genes *MYC2*, *MYC3*, and *MYB76*, amino acid elongation genes *BCAT3*, *BCAT4* and *MAMI*, core structure genes *CYP83A1*, *UGT74B1* and *SURI*, and side chain modification genes *FMO GS-OXI* and *AOP2*-like (Table 4.1). In addition to identifying lines with putative candidate genes for reduced glucosinolates, we also performed wet lab analysis on these 23 lines. We observed that D3-N13P3 and E5-133P2 lines carrying fixed homozygous mutations in *MYC3* resulted in a 38-46% reduction in glucosinolates compared to the wild-type. Line E3196 carrying

fixed homozygous mutations in *AOP2* resulted in a ~92% reduction compared to the wild-type, and it has been characterized in a recent pennycress domestication report (Chopra et al., 2020). Many of the putative candidate lines or target genes were in the heterozygous condition and require further genetic characterization. To further confirm the utility of this mutant resource for identifying candidate genes for reduced glucosinolate mutants, we selected two mutant lines of *MYC3* (D3-N13P3; E5-133P2) and one mutant line of *CYP83A1* (A7-66) for further characterization.

Table 4.1 - Summary of mutations in the candidate genes involved in glucosinolate biosynthesis based on Arabidopsis.

Category	Line	Gene Names	Amino Acid Change	Zygoty	Wet Lab Values $\mu\text{mol/g}$
Wild-type	MN106	-	-	-	113.4
Transcription Factors	A7-111	<i>MYC2</i>	Arg456Cys	Homozygous	108.7
	E5-359P3	<i>MYC3</i>	Ala19Val	Heterozygous	57.7
	D3-N13P3	<i>MYC3</i>	Trp66*	Homozygous	62.2
	E5-133P2	<i>MYC3</i>	Gly135Glu	Homozygous	71.2
	D3-22	<i>MYB76</i>	Val412Ile	Heterozygous	97.4
Amino Acid Biosynthesis	D3-N13P3	<i>BCAT4</i>	Ala306Val	Heterozygous	62.2
	E5-296P2	<i>BCAT3</i>	Gly396Glu	Heterozygous	34.2
	D3-N57P3	<i>BCAT3</i>	Val320Met	Heterozygous	90
	E5-543	<i>MAMI</i>	Arg158Lys	Heterozygous	87.6
	E5-359P3	<i>MAMI</i>	Ala11Thr	Heterozygous	57.7
Core Structure biosynthesis	A7-66	<i>CYP83A1</i>	Glu417Lys	Heterozygous	105.7
	A7-184	<i>CYP83A1</i>	Leu15Phe	Heterozygous	94.1
	2019-M2-122	<i>CYP83A1</i>	Gln245*	Heterozygous	NA
	E5-549	<i>UGT74B1</i>	Thr441Ile	Heterozygous	92.2
	A7-188	<i>SUR1</i>	Gly28Ser	Heterozygous	79.9
	A7-229	<i>SUR1</i>	Arg153*	Heterozygous	82.3
	E5-337P2	<i>SUR1</i>	Asp386Asn	Heterozygous	65.1
	E5-282P2	<i>SUR1</i>	Glu836Lys	Heterozygous	111.8
	E5-484P5	<i>FMO GS-OXI</i>	Val13Met	Heterozygous	90.7

Secondary Side Chain Modification	A7-11	<i>FMO GS-OXI</i>	Arg27Lys	Homozygous	104.6
	E5-356P5	<i>FMO GS-OXI</i>	Arg1061His	Heterozygous	88.3
	A7-129	<i>AOP1</i>	Gly380Arg	Heterozygous	78.8
	D3-N10P5	<i>AOP1</i>	Glu133Lys	Heterozygous	94.2
	E3196	<i>AOP2</i>	Gly97Arg	Homozygous	9

4.3.3. Genetic characterization of candidate genes in pennycress glucosinolate biosynthesis pathway

We identified three independent alleles in the transcription factor gene *MYC3* (Table 4.1). Two alleles, *myc3-2* and *myc3-3* were homozygous, and one allele, *myc3-1*, was heterozygous. The mutation in line E5-359P5 (*myc3-1*), resulted in an amino acid change from alanine to valine at the 19th position. The mutation in line D3-N13-P5 (*myc3-2*), resulted in a stop codon instead of tryptophan at the 66th position. The mutation in line E5-133-P3 (*myc3-3*), resulted in an amino acid change from glycine to glutamic acid at the 135th position (Supplementary File 1 – Appendix 1). While it exhibited a low-glucosinolate phenotype, we excluded line E5-359-P5 from further characterization because it also carried a mutation in the side chain elongation gene *MAMI*. Identification of two homozygous independent *myc3* alleles resulting in reduced seed glucosinolates provided evidence that *MYC3* was likely a causative candidate for the low glucosinolate phenotype (Figure 4.3).

At-MYC3	DDASAAA...IESAGENWTY.....LISGGTGVSD
Ta-MYC3	DDNASAA...IESAGEGWTY.....LISGGTGVSD
Ta-myc3-1	DDNASAV...IESAGEGWTY.....LISGGTGVSD
Ta-myc3-2	DDNASAA...IESAGEG*
Ta-myc3-3	DDNASAA...IESAGEGWTY.....LISGGTEVSD

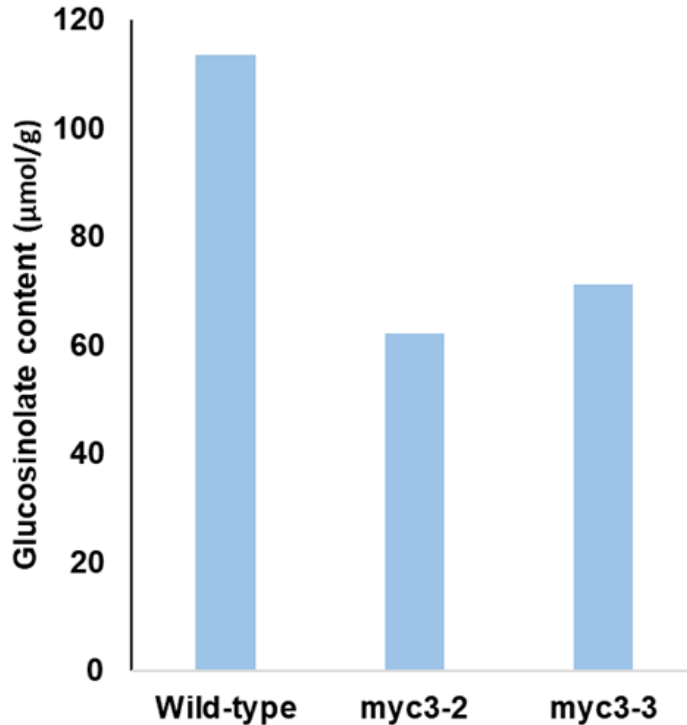


Figure 4.3 - A) Amino acid comparisons of Arabidopsis and Pennycress *MYC3* gene along with the mutant alleles identified in the Table 4.1. B) Glucosinolate content of wild-type, *myc3-2* and *myc3-3*.

To further confirm the allele effect, we generated an F1 plant by crossing the wild-type and *myc3-2* mutant. F1 plants were verified for the heterozygous *myc3-2* allele and F2 seeds were collected for further analysis. F2 seeds derived from this population were planted in the field during the Fall of 2018, and F3 seeds were harvested from individual plants. A total of 18 randomly selected progenies were scanned using NIR, and seed DNA was used to identify respective genotypes. Genotypic segregation patterns were similar to the expected ratios of an F2 population. The glucosinolate values associated with the mutant allele were ~20% lower than the corresponding wild type (Table 4.2). A

goodness-of-fit test based on a χ^2 analysis of genotypic segregation ratios revealed that the observed values were statistically similar to the expected values. The finding of two *myc3* allelic mutants with decreased glucosinolates and the co-segregation of *myc3-2* with a reduced glucosinolate phenotype provide strong support that these mutations are causative.

Table 4.2 - Summary of F2 population segregating for the *myc3-2* allele.

Genotype	Mean	SD	# of samples
Wild-type	96.15 ^{ab}	13.61	4
Heterozygous	94.77 ^a	13.17	10
Mutant	77.62 ^b	7.134	4

A pairwise Tukey test suggested that wild-type and heterozygous pools are statistically different from the mutant pool at a p-value of 0.10. Letters represent that these values are statistically significant and belong to two separate groups.

We identified three independent alleles in the glucosinolate core structure biosynthesis gene *CYP83A1*. All of these mutations were heterozygous. Line A7-184 (*cyp83a1-1*) carried a mutation that changed the amino acid leucine to phenylalanine at the 15th position. Line 2019-M2-122 (*cyp83a1-2*) carried a mutation that led to stop codon instead of glutamine at the 245th position, and A7-66 (*cyp83a1-3*) carried a mutation that changed glutamic acid to lysine at the 417th position (Supplementary File 1 – Appendix 1). The *cyp83a1-2* mutant, which harbored a stop codon, did not produce a viable homozygous plant in the segregating population (Figure 4.4B). Similar results have been observed in *Arabidopsis* (Naur et al., 2003).

At-CYP83A1	AYMKECFERQ
Ta-CYP83A1	KYMMDCFERQ
Ta-cyp83a1-2	KYMMDCFER*



Figure 4.4 - A) Comparison of amino acid sequences of Arabidopsis and Pennycress *CYP83A1* along with the *cyp83a1-2* allele. B) Comparison of wild-type and mutant plants obtained from segregating populations of *cyp83a1-2* allele.

To confirm the mutation effect in the *cyp83a1* mutants, we selected *cyp83a1-3*, which had a heterozygous mutation in the PERF motif (Figure 4.5A). Previously, we developed a method that allows for parallel assessment of genotype and fatty acids on a single pennycress seed. In this study, we adopted a similar strategy using a glucosinolate assay instead of a fatty-acid assay. Briefly, we extracted glucosinolates from a single seed using 80% methanol and used the method described above to inhibit the breakdown by myrosinase and estimate glucosinolate levels using a spectrophotometer. The remaining seed meal from each sample was subject to DNA extraction followed by genotyping. For the modified procedure described above, we selected 24 individual M5 seeds from a heterozygous *cyp83a1-3* M4 pool. Based on the genotyping results, we found 10 single seeds with the wild-type allele, 8 single seeds with mutant alleles, and 6 seeds with

heterozygous alleles. The segregation observed in M5 seeds suggested that the pool was getting fixed for one or other alleles at this generation. Glucosinolates for the wild-type pool averaged $138.00 \pm 12.22 \mu\text{mol/g}$, the heterozygous pool averaged $136.00 \pm 15.17 \mu\text{mol/g}$, and the mutant pool averaged $113.00 \pm 18.54 \mu\text{mol/g}$. These results suggested that the glucosinolate content of the homozygous mutant was $\sim 18\%$ lower than the wild type within the population (Figure 4.5B). A pairwise Tukey test suggested that wild-type and heterozygous pools are statistically different from the mutant pool at a p-value of 0.05.

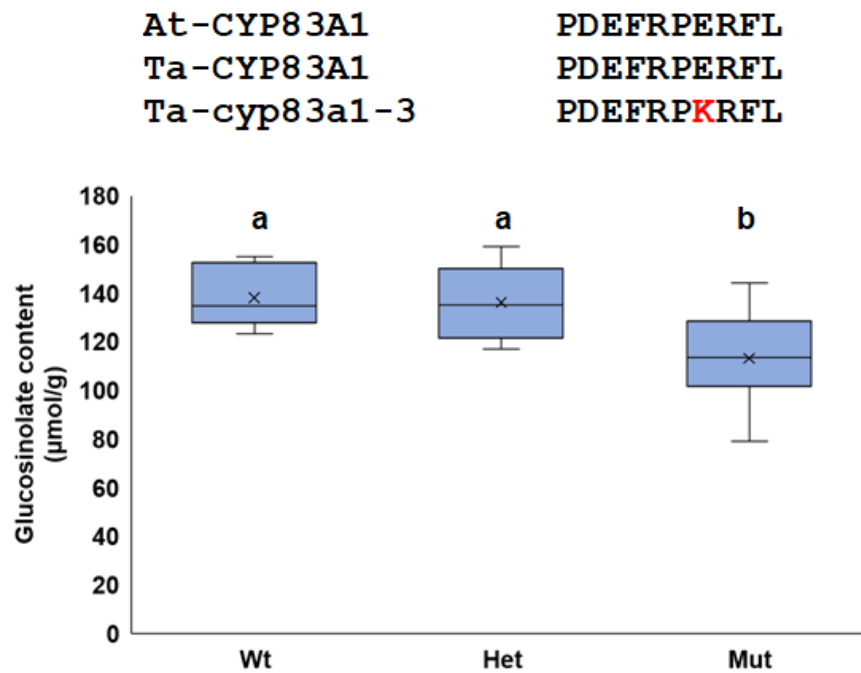


Figure 4.5 - A) Amino acid comparisons of Arabidopsis and pennycress *CYP83A1* gene along with the *cyp83a1-3* mutant allele identified in the Table 4.1. B) Glucosinolate content of wild-type, heterozygous and mutant seed pools of *cyp83a1-3* allele. Letters above the box plot show that wild-type and heterozygous pools belong to one group and mutant pools to a different group based on pairwise Tukey tests.

To further confirm the heritability of this trait we selected three seedlings for each wild-type and the mutant from the M₄ pools and grew the plants until maturity. We performed

wet-lab analysis on three independent wild-type and mutant progenies for glucosinolate content and found homozygous mutant progenies showed 10.75 ± 0.24 % reduction compared to the wild-type (Table 4.3). These results confirmed the low-glucosinolate trend predicted in the single seed analysis and provided more evidence for the causative effect of the *cyp83a1-3* allele.

Table 4.3: Glucosinolate content determined using wet-lab method in the wild-type and mutant progenies of *cyp83a1-3* mutants (n=3).

Genotype	Mean	SD
CYP83A1 (Wild-type)	124.1 ^a	5.2
<i>cyp83a1-3</i> (Mutant)	110.8 ^b	6.5

A pairwise tukey test suggested that wild-type pools are statistically different from the mutant pool at a p-value of 0.10. Letters represent that these values are statistically significant and belong to two separate groups.

In Arabidopsis, it has been shown that *MYC3* works with *MYC2* and *MYC4* to regulate jasmonic acid and glucosinolate biosynthesis pathways (Schweizer et al., 2013).

CYP83A1 is involved in oxime metabolism of core structure biosynthesis in the pathway (Naur et al., 2003). To understand the effect of these mutations on other genes involved in pennycress glucosinolate production, we performed qPCR analysis on three pathway genes, including the amino acid side chain elongation gene *MAMI*, the core structure biosynthesis gene *CYP83A1*, and the secondary side chain elongation gene *AOP2*-like.

The *myc3-2* mutants showed significant reductions in expression compared to the wild type, likely due to the complete *lof* mutation in the *MYC3* gene. The reductions in expression for each of these genes included *MAMI* (0.98-fold reduction), *AOP2*-like (0.82-fold reduction) and *CYP83A1* (0.78-fold reduction) (Figure 4.6A). A student t-test

comparing the fold change in expression between the mutant and wild-type was statistically significant at a p-value of 0.05. We expected the *cyp83a1-3* mutant to exhibit reductions in gene expression due to the mutation in the PERF domain. Mutants exhibited reduced expression compared to the wild type, including *MAM1* (0.16-fold reduction), *AOP2*-like (0.22-fold reduction) and *CYP83A1* (0.14-fold reduction) (Figure 4.6B). Although expression of these genes was reduced in the mutant plants, a student t-test comparing the fold change of wild-type and mutants suggested that these differences were not statistically significant at a p-value of 0.05. Overall, a combination of genetic, biochemical, and functional characterization of *MYC3* and *CYP83A1* confirm their role in glucosinolate biosynthesis in pennycress.

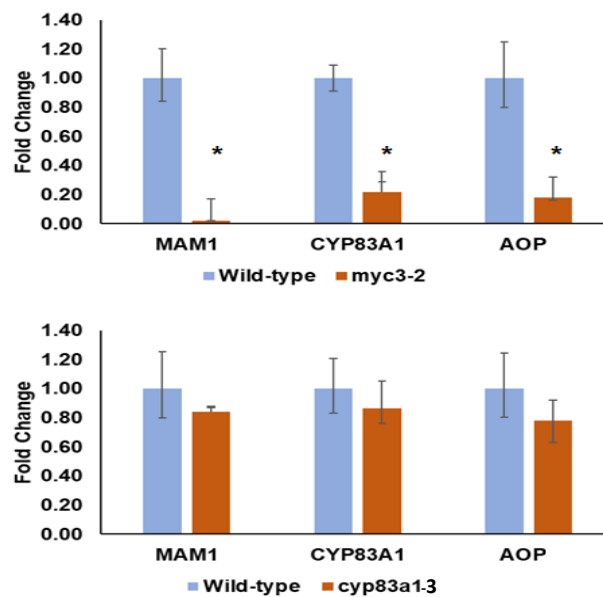


Figure 4.6 - A) Expression analysis of pennycress *MYC3* gene on the wild-type plants and *myc3-2* mutants. B) Expression analysis of pennycress *CYP83A1* gene on the wild-type plants and *cyp83a1-3* mutants. These values represent fold changes derived from Ct values of the wild-type and mutant samples and actin was used as a reference gene. Each of the experiments had 3 biological and 3 technical replicates. * - represents fold change of wild-type and mutants are statistically significant at a p-value of 0.05.

4.4 Discussion

Improving meal quality is critical to unlock the food use potential for pennycress.

Thousands of pounds of seed meal (i.e. press-cake) are generated as a waste stream from the oilseed crushing process. Given its rich protein content, pennycress seed meal could be positioned as an animal feed additive or, potentially, a plant-based protein source for human food, similar to byproducts of comparable oilseeds. However, high levels of anti-nutritional glucosinolates render the seed meal unpalatable. Thus, improving meal quality valorizes the major waste stream of the crushing process, which improves the value of the overall pennycress system.

Identifying pennycress mutants with desirable reductions in glucosinolates could lead to the development of low glucosinolate pennycress lines that meet regulatory standards. A recent report on pennycress domestication highlighted a mutation in the *AOP2*-like gene that reduces glucosinolate content to very low levels and exhibits a pleasant “nutty” flavor (Chopra et al., 2020). While the *AOP2-like* gene is an important candidate for pennycress improvement, we are seeking to identify additional mutations that confer desirable reductions in seed glucosinolates. This study evaluated additional mutants with reduced glucosinolates and identified putative mutations in these lines. We found that we could effectively use NIRS to select for lines with reductions in seed glucosinolates as shown previously (Chopra et al., 2019). We also found that well-studied glucosinolate genes in *Arabidopsis* likely play similar roles in pennycress. Notably, some pennycress lines, such as E5-444P1, exhibiting a low glucosinolate phenotype (24 $\mu\text{mol/g}$) did not carry a mutation in an obvious glucosinolate pathway gene or pathway regulator. These

reduced glucosinolate lines may be valuable resources to further understand and dissect glucosinolate biosynthesis and regulatory mechanisms in pennycress and other brassicas. In the future, we can generate a backcross F₂ population from these lines and identify likely candidates using bulk-segregant analysis (BSA) if the trait segregates in Mendelian ratios (Fekih et al., 2013).

We used a forward genetics approach to identify low-glucosinolate mutants. In parallel, we used a reverse genetics approach to identify alleles in genes regulating pennycress glucosinolate biosynthesis based on prior work in *Arabidopsis* (Sønderby et al., 2010). We successfully identified mutations in many of the predicted genes. These mutations were present in transcription factors, amino acid elongation enzymes, core structure genes and secondary side chain modification genes. To increase the availability of reduced glucosinolate candidates for pennycress improvement and to confirm the utility of the pennycress mutant gene index for reduced glucosinolate traits, we validated two of the alleles identified in this study: one from a transcription factor - *MYC3*, and one from core structure biosynthesis - *CYP83A1*.

In *Arabidopsis*, it has been shown that several transcription factors regulate the glucosinolate biosynthesis pathway. The transcription factors *MYB28*, *MYB76*, and *MYB29* closely regulate genes in the aliphatic glucosinolate biosynthesis pathway (Gigolashvili et al., 2007; Ida Elken Sønderby et al., 2007) whereas the transcription factors *MYB51*, *MYB34*, and *MYB122* closely regulate indole glucosinolate biosynthesis in *Arabidopsis* (Frerigmann & Gigolashvili, 2014). In addition, the transcription factors

MYC2, *MYC3* and *MYC4* form a complex and interact with glucosinolate related transcription factors to maintain the flux of secondary metabolites (Burow et al., 2015). Glucosinolate reductions have been observed in *myc234* triple mutants in Arabidopsis (Schweizer et al., 2013) and not during reduced expression of individual genes. This suggests that *MYC2*, *MYC3*, and *MYC4* may have redundant functions.

In this study, we identified one allele in *MYC2*, three alleles in *MYC3*, and no mutations in *MYC4*. We selected mutant alleles in *MYC3* for further validation. Two of the homozygous mutants showed significant reduction compared to the wild-type plants (Figure 4.2). This allelic variation supported the role of *MYC3* in pennycress glucosinolate production. We further characterized the *myc3-2* mutant, which possessed a stop codon, by performing genetic expression analysis. Our expression analysis comparing the wild type to the *myc3-2* mutant showed significant reductions in *MAMI*, *CYP83A1* and *AOP2*-like. Similar strong reductions in expression have been observed in the *myc234* Arabidopsis triple mutant (Schweizer et al., 2013). Overall, these results suggest the role of *MYC3* in pennycress glucosinolate biosynthesis, and future work is required to characterize *MYC2* and *MYC4* genes.

Previous studies have suggested that *MYC2*, *MYC3* and *MYC4* mutants can influence seed protein content (Gao et al., 2016). We did not observe a significant effect on the total protein and oil content predicted using NIRS in the *myc3-2* mutant (Table 4.4). Future work may assess the influence of these transcription factors on pennycress seed

size and protein composition as these traits may be crucial for increasing the value of the seeds.

Table 4.4 - NIRS predictions of F2 population segregating for *myc3-2* allele.

Genotype	Glucosinolates	Protein	Oil
Wild-type	96.15 ^{ab}	23.19 ^a	37.02 ^a
Mutant	77.62 ^b	23.66 ^a	37.72 ^a
Heterozygous	94.77 ^a	22.97 ^a	36.76 ^a

A pairwise tukey test suggested that wild-type and heterozygous pools are statistically different from the mutant pool at a p-value of 0.10. Letters represent that these values are statistically significant and belong to two separate groups.

Several enzymes have been identified that regulate the core-structure of the glucosinolate biosynthesis pathway in Arabidopsis. CYP79F1 is the first aldoxime-forming enzyme in the biosynthesis of aliphatic glucosinolates (S. Chen et al., 2003) followed by the CYP83A1 enzyme, which is involved in oxime metabolism (Naur et al., 2003). UGT74B1 and SUR1 are two other enzymes involved in this pathway (Boerjan et al., 1995; Douglas Grubb et al., 2004; Mikkelsen et al., 2004). In this study, we identified three alleles in *CYP83A1*, one allele in *UGT74B1* and four alleles in *SUR1*. Mutations in core structure genes can have negative impacts on plant development and fitness due to their involvement in auxin homeostasis (Douglas Grubb et al., 2004; Hemm et al., 2003; Mikkelsen et al., 2004; Naur et al., 2003). To test if mutations in core structure genes would result in a similar phenotype, we selected three alleles from the *CYP83A1* gene. All of these mutants were heterozygous, and we selected two alleles for further characterization.

The *cyp83a1-2* mutant harbored a mutation that led to a stop codon. A segregating population of *cyp83a1-2* alleles was grown to test for the low glucosinolate phenotype and for further evaluation. Unfortunately, homozygous mutant plants did not produce flowers (Figure 4.4). Similar observations have been made in Arabidopsis, and it has been hypothesized that mutations in *CYP83A1* result in strong changes in the metabolite profile of the plants (Hemm et al., 2003; Naur et al., 2003). Fortunately, *cyp83a1-1* and *cyp83a1-3* most likely experienced partial *lof* in *CYP83A1* based on the seed glucosinolate phenotype, and characterization of these mutants helped confirm the role of pennycress *CYP83A1* in pennycress glucosinolate production. We found that the mutation in the *cyp83a1-3* allele was present in the PERF motif, which is involved in locking the heme pocket into position and to assure stabilization of the conserved core structure (Hasemann et al., 1995). Even though the mutation occurs at the end of the protein, mutations found in crucial motifs may influence glucosinolate phenotypes in pennycress. Overall, these results suggest a role of *CYP83A1* in pennycress glucosinolate biosynthesis, and future work is required to characterize other pathway genes.

As discussed previously in the pennycress domestication report, gene-editing approaches could create reduced glucosinolate mutants based on translational research (Chopra et al., 2020). In this study, using a mutagenesis approach helped find new targets such as *MYC3* that might not be of interest based on phenotypes observed in Arabidopsis. The mutagenesis approach also created a partial loss-of-function mutation in *cyp83a1-3* rather than fully knocking out the gene and potentially harming plant fitness.

The levels of seed glucosinolates in *myc3* and *cyp83a1* mutants were not reduced to levels required to be comparable to canola or pennycress *aop2*-like mutants. Further studies on the candidate alleles such as *MAMI* and *FMO GS-OXI* found in this study would help identify and isolate mutants with a reduced glucosinolate phenotype. Combining mutant alleles of different genes in the future could allow for stronger reduction in glucosinolate levels and also provide resources for understanding gene interactions. Overall, these results indicate the success of using an EMS mutagenesis approach to identify candidate genes for pennycress glucosinolate biosynthesis. We believe this strategy of identifying the genes regulating glucosinolate and other metabolite biosynthesis in pennycress will be effective and will help us advance genetics in pennycress and many other Brassicas.

4.5 Future perspectives

Combining the *myc3* and *cyp83a1* low glucosinolate mutants with other beneficial traits can potentially lead to a viable, food grade pennycress variety. More work is needed to understand exact mechanisms underlying glucosinolate accumulation in pennycress. The work presented in this study is largely based on previous research in the model plant *Arabidopsis thaliana*, a close relative to pennycress (Dorn et al., 2015). Similar results have been observed in *Arabidopsis*, yet there are potentially significant differences. For example, mutations in *MYC3* reduce glucosinolates in *Arabidopsis* as part of the triple mutant *myc234* (Schweizer et al., 2013). These mutants also experience disruptions in crucial development pathways, such as the jasmonic acid pathway, which can decrease plant fitness. In contrast, the *myc3* pennycress mutant experiences a significant decrease in glucosinolates without decreasing plant fitness. This indicates that *MYC3* potentially

plays a larger role in glucosinolate accumulation than previously thought. Additionally, altering glucosinolate biosynthesis and regulation may cause shifts in production of other metabolites (Slaten et al., 2020). For example, amino acids that would normally participate in glucosinolate biosynthesis may be diverted to produce other compounds. This could influence end-uses for the crop. Additional work is also needed to understand how low glucosinolate mutants perform in on-farm production scenarios. Since glucosinolates provide a defensive function, reducing them may render the plants more susceptible to pest.

4.6 Conclusion

Using a forward and reverse genetics approach, over 15,000 mutant pennycress lines were assessed for reduced glucosinolate levels. Low-glucosinolate mutants underwent whole genome sequencing to identify putative candidate genes responsible for glucosinolate accumulation. Lines carrying mutations in the transcription factor *MYC3* and the core structure biosynthesis gene *CYP83A1* exhibited a reduced glucosinolate phenotype compared to wild plants; however, these reductions did not reach the canola threshold of 30 $\mu\text{mol/g}$. Expression analyses revealed that other genes involved in glucosinolate biosynthesis and regulation were downregulated when these mutations were present, indicating *MYC3* and *CYP83A1* play a role in glucosinolate accumulation. While more work is needed to understand exact genetic mechanisms underlying glucosinolate accumulation in pennycress, these genes are potential candidates to produce a viable food grade pennycress in combination with other beneficial genes.

4.7 Relevance to the nutrition ecology framework and the food system

This project primarily aligned with the environmental and health dimensions of the nutrition ecology framework. The environmental dimension encompasses many factors, like the relationship between agricultural production, biodiversity, resource use, and climate change. Pennycress is being developed to contribute to biodynamic, regenerative agriculture as a cash cover crop. Given the potential environmental benefits of including pennycress in conventional crop rotations, this project clearly aligns with the environmental dimension of the nutrition ecology framework. The health dimension encompasses food quality, and this project largely focused on identifying genetic targets that could improve food quality by reducing anti-nutritive compounds. Improving food quality of the seed meal contributes to the valorization of the pennycress biproducts, thus improving the economic output of the pennycress supply chain. Therefore, the project also somewhat aligns with the economic dimension of the nutrition ecology framework. Overall, developing crops that produce environmental benefits and that can potentially serve as food sources contributes to the development of more sustainable food systems.

Chapter 5: Understanding on-farm production economics of canola reveals potential economic impact of field pennycress

Abstract

Field pennycress (*Thlaspi arvense L.*) is a winter annual oilseed undergoing development as a cash cover crop for the Upper Midwest. The crop provides ecosystem benefits as a continuous living cover during the winter fallow period between corn harvest and soybean planting. Current work aims to optimize the agronomic management of the crop and to improve the chemical composition of oil and meal constituents to increase potential market value. More work is needed to understand the supply chain economics of this crop. Therefore, this study uses an enterprise budget modeling approach to estimate the on-farm production economics of pennycress in USD on a per acre basis. Given its close relationship to conventional oilseeds, we use on-farm financial data from canola-producing farms to estimate price and expenses for pennycress. We estimate breakeven yield at the 10-year average price received by canola producing farmers in Minnesota and North Dakota between 2008-2017. We determine that pennycress must yield 1,495 pounds per acre for producers to break even. We estimate net return of three different yield scenarios based on existing literature. Based on yields reported in the literature, we estimate net returns ranging from a net loss of \$152.9 per acre to a net return of \$97.74 per acre. Incorporating pennycress into crop rotations has been reported to influence soybean yield, so we then estimate the economic impact of soybean yield reductions ranging from 5-30%. We find that soybean yield reductions potentially result in net losses. Finally, we estimate net returns of the corn-pennycress-soybean crop rotation. Based on all pennycress and soybean scenarios generated in this study combined with the average net return for corn, we determine that the return for the three crop system ranges from a net loss of \$136.98 to a net return of \$220.12 per acre. This is one of the first studies using on-farm production data to estimate the potential economic output of pennycress. We show a wide range of net returns for pennycress as a standalone crop and for the three-crop system. More work is needed to understand actual production values and to estimate potential economic benefits of ecosystem services provided by pennycress.

5.1 Introduction

Field pennycress (*Thlaspi arvense L.*) is a wild, winter annual oilseed undergoing development as a cover crop for the Upper Midwest (Sedbrook et al., 2014). As a winter annual, pennycress is planted in the fall and matures in the early spring. The crop is particularly cold tolerant and winter hardy, which suits the harsh winters of the Upper Midwest. Therefore, it is being developed to fit within the conventional corn-soy rotation that covers over 80 million acres in the Upper Midwest (N.R. Jordan et al., 2016). Figure 5.1 illustrates a potential corn-pennycress-soy crop rotation. Planted following corn harvest and harvested prior to soybean planting, pennycress provides continuous living ground cover on land traditionally left barren in conventional crop systems (Phippen & Phippen, 2012). During the fallow period, pennycress contributes to bio-dynamic, regenerative agriculture by sequestering key soil nutrients, limiting soil erosion, preventing water pollution, suppressing weeds, providing a food source to early spring pollinators, and ultimately increasing biodiversity (Eberle et al., 2015; Johnson et al., 2015; Thom et al., 2018; Weyers et al., 2019). In other words, pennycress enhances agroecosystems without displacing land used for conventional crops.

Recent genetic advances have facilitated the discovery of critical traits required to improve on-farm production of pennycress (Chopra et al., 2020). These traits, considered part of the domestication syndrome, include early flowering, uniform maturity, reduced seed pod shatter, and other agronomically important traits (Chopra et al., 2020). In a short period of time, pennycress has advanced from a wild, weedy species to a potentially viable cash cover crop.

As a historically wild, weedy plant, pennycress faces challenges to adoption as a cash cover crop, including a lack of clearly defined markets. Cover crops face barriers to adoption because they often add expenses to farm management without provided a return (Myers et al., 2019). Current works aims to develop pennycress as a cash cover crop for the bioeconomy (N.R. Jordan et al., 2016). Beyond agroecosystem services, pennycress produces seeds rich in oil (~35%), protein (~20-25%), and fiber (~15%) (Evangelista et al., 2012). Recent studies have shown that pennycress oil is a suitable feedstock for industrial applications, such as bio-based fuels (Boateng et al., 2010; Moser, Knothe, et al., 2009; Moser, Shah, et al., 2009). Other reports indicate that pennycress seed meal is potentially suitable as a biofumigant (Vaughn et al., 2005). These applications have garnered interest for two reasons: 1) pennycress might improve the environmental impact of agriculture and 2) bio-based industrial feedstocks can help transition away from fossil fuels. While these end-uses may contribute to the bioeconomy, further valorizing the oil and meal components could unlock other economic opportunities.

In addition to industrial end-uses, current work aims to develop pennycress for food use. Wild pennycress is unsuitable for food and feed use due to the presence of toxic compounds in the oil and seed meal. Wild pennycress oil is rich in erucic acid, which is associated with heart disease in animal models (Hulan et al., 1976). Meanwhile, seed meal contains high levels of glucosinolates, pungent, bitter compounds, which render the seed meal unpalatable and produce negative effects on the thyroid (J. M. Bell, 1993; Chopra et al., 2019). As part of the domestication process, researchers discovered lines

harboring food grade qualities (Chopra et al., 2020). These advanced pennycress lines exhibit oil profiles and seed meal qualities, similar to canola.

Canola is the second most consumed food oil and protein meal in the United States, trailing only soybean in both categories. In 2019, the United States consumed over 4.2 billion pounds of canola oil and 4.8 million tons of canola meal (USDA-ERS, 2020). In contrast, the US produced 1.6 billion pounds of oil and 1 million pounds of meal during this time frame. Most of the US canola supply arrives from Canada. Developing food grade pennycress and deploying it at scale could provide a new source of food oil and seed meal while improving the environmental impact of conventional crop systems. Deploying pennycress at scale requires further understanding of production economics.

Similar to other cover crops, there is a limited understanding of pennycress production economics. While cover crops provide environmental benefits, it is often difficult to calculate the cover-crop-return to the bottom line of a farm. According to the Sustainable Agriculture Research & Education program (SARE), cover crops are most profitable when herbicide-resistant weeds are a problem, cover crops are grazed, soil compaction is an issue, cover crops are used to speed and ease the transition to no-till, soil moisture is at a deficit or irrigation is needed, fertilizer costs are high or manure nutrients need to be sequestered, or incentive payments are received (Myers et al., 2019). Since pennycress seeds have the potential to fit into end use markets, the crop may be more profitable compared to conventional cover crops. There is limited economic research on pennycress production.

Mousavi-Avval and Shah (2020) performed a techno-economic assessment to evaluate the pennycress production, harvest, and post-harvest logistics for renewable jet fuel (Mousavi-Avval & Shah, 2020). Here, researchers mapped the production system including land use, equipment and machinery needs, labor requirements, consumables, post-harvest logistics, and commercial scale manufacturing activities for an Ohio-based biorefinery. They projected that 41-63 thousand hectares with an annual harvest of 90-115 thousand tons are required to supply a single biorefinery (Mousavi-Avval & Shah, 2020). Total production costs ranged from \$170-\$230 per ton, which is considerably less than comparable oilseeds, like canola, camelina, and carinata (Mousavi-Avval & Shah, 2020). Other studies have evaluated production economics based on field trials. Ott and colleagues compared the economics of a double crop system (e.g. winter oilseeds and soybeans) to a monocrop system (e.g. soybeans alone) (Ott et al., 2019). The report found that economic output of the double crop system never surpassed the monocrop system, and in some cases, produced a net loss. Similarly, Cubins found that the pennycress-soybean system was less economical than the monocrop system (Cubins, 2019). In both cases, the negative economic implications were linked to low yielding pennycress varieties, lack of optimized agronomic management practices, and increased expenses of producing two crops. Clearly, more work is needed to understand to pennycress production economics.

Given the similarity between the two crops, an understanding of canola economics might provide insight into the potential economic impact of field pennycress. Here, we produce an enterprise budget based on average on-farm production economic data for canola in

two Upper Midwest states, North Dakota and Minnesota. We model pennycress production scenarios under different yield conditions based on yields reported in the literature to understand the impact of yield on return. We then calculate the breakeven yield based on a 10-year average canola price received by farmers. We then model the potential economic impact of soybean yield reductions on soy net return. Finally, we estimate the net return of the corn-pennycress-soybean system based on several different yield scenarios. We ultimately conclude that pennycress economics vary greatly depending on yield, and that potential soybean yield reductions are critical to this system.

5.2 Methods

Enterprise budgets were constructed for pennycress, soybean, and corn to model individual crop production economics and production economics of the three-crop system. These enterprise budgets summarize returns and expenses of farming practices on a per acre basis in USD. Key components of the budget include yield, value per unit, total return per acre, crop insurance per acre, other crop income, and gross return per acre. Direct expenses are those that directly contribute to growing a crop. These expenses include seed, fertilizer, crop chemicals, crop insurance, fuel & oil, custom hire, hired labor, land rent, operating interest, and miscellaneous costs. Overhead expenses are those involved in operating a business. These include custom hire, hired labor, machinery leases, building leases, farm insurance, utilities, dues & professional fees, interest, and machine and building depreciation costs. Given the similarity between pennycress and canola, financial production data, including price, direct, and overhead expenses, were used to model the economics of pennycress production. Budgets for three different yield

scenarios were created using low, middle, and high yielding values reported in the literature.

Farm level data were collected from FINBIN (<https://finbin.umn.edu/>), a farm financial database that collects information from producers using the FINPACK (<https://finpack.umn.edu/>) financial planning software. FINBIN summarizes farm-level production data from thousands of producers throughout the Midwest and provides benchmark financial information for producers, educators, lenders, and other agricultural professionals (*About FINBIN*, 2020). Farm-level data were collected from the following canola-producing regions: North Dakota North Central Farm and Ranch Business Management, North Dakota South Central Farm and Ranch Business Management, North Dakota Western Missouri Slope Farm and Ranch Business Management, Minnesota State College & University North, and Minnesota State College & University Red River Valley. Data were collected and averaged based on the 2008-2017 period to create an average enterprise budget for canola, corn, and soybean. Average, minimum, and maximum values are reported for this time frame.

The pennycress enterprise budget includes three different yield scenarios based on yields reported in the literature. Hedging gains/losses per acre, crop insurance per acre, and other crop income per acre are not reported in the pennycress enterprise budget. Net return was calculated for each crop by subtracting direct and overhead expenses from gross return. Average, minimum, and maximum returns are reported for each crop.

A breakeven yield analysis was performed to understand pennycress yield required for return to equal expenses. To determine the breakeven yield, average canola expenses of \$287.44 were divided by the average canola price of \$19.22 per cwt.

Including pennycress in crop rotations potentially impacts soybean yields. It has been reported that soybeans planted following pennycress experience yield losses up to 30% (Johnson et al., 2015; Hoerning et al., 2020). Therefore, yield loss scenarios ranging from 5%-30% were generated to determine the impact of yield loss on net return. Yield losses were calculated using the 2008-2017 average soybean yield as a base. Price remained constant based on the 2008-2017 average. Average insurance, hedging gains/losses and other income per acre remained constant. Average direct and overhead expenses also remained constant. Net return was calculated by subtracting expenses from gross return in each scenario.

To understand the potential economic impact of the three-crop system, we generated different scenarios showing combined net return (gross return minus direct and overhead expenses) with varying pennycress and soybean yields. In each scenario, corn net return was held constant at the 2008-2017 average return of 50.52 per acre. This approach generated a range of net returns at each level. All values are reported on a per acre basis in USD.

5.3 Results

5.3.1 Canola enterprise budget

Table 5.1 summarizes the canola enterprise budget values over the 2008-2017 period. Values are reported on a per acre basis in USD. During this timeframe, canola yield averaged 16.99 cwt per acre, with an average value of \$19.22 per cwt. The lowest price during this period was \$15.87 per cwt while the highest price was \$28.43 per cwt. The average gross income per acre totaled \$340.34 with a range of \$294.87-429.07. Average direct expenses totaled \$244.81 per acre with a range of \$210.73-284.55. Average overhead expenses totaled 42.63 per acre with a range of \$29.04-55.92. Average net return per acre ranged from -\$29.77-101.95, with an average of \$52.90.

5.3.2 Pennycress yield scenarios and breakeven yield

To determine the potential impact of yield on profitability, we generated three different scenarios based on yields reported in the literature and on-farm trials. Values are reported on a per acre basis to provide a practical example for producers. Pennycress has been reported to potentially yield between 1100-2250 kg per ha (Sedbrook et al., 2014). This translates to roughly 979.76-2004.05 pounds per acre. Meanwhile, the Agricultural Marketing Resource Center reports that yields of 700-900 pounds per acre are more likely in farm production scenarios (*Pennycress*, 2018). While some studies report even lower yields (e.g. as low as 100-200 kg per ha (Dose et al., 2017; Johnson et al., 2015)), our model uses the maximum projection of 2004.05 pounds per acre (2250 kg per ha) and the minimum projection of 700 pounds per acre along with a middle projection of 979.76 pounds per acre (1100 kg per ha).

Table 5.2 summarizes potential pennycress budgets at three different yield estimates. At the average canola price of \$19.22 per cwt, our top-yielding pennycress scenario

generates \$385.18 gross return per acre. Assuming combined direct and overhead expenses of \$287.44 based on canola, net return equals \$97.74. The lowest-yielding pennycress scenario (700 lbs. per acre) generates \$134.54 gross return per acre, while return over expenses results in a loss of \$152.90. The middle yielding scenario (979.76 lbs. per acre) generates \$188.31 gross return per acre while return over expenses results in a loss of \$99.13. Given the wide variation between yield and potential returns, we calculated the yield required for gross return to counter estimated expenses at the 10-year average price of \$19.22 per cwt, i.e. the breakeven yield. At a price of \$19.22 per cwt, our model predicts a breakeven yield of 1,495 pounds per acre, assuming expenses are similar to canola.

5.3.3 Pennycress influence on soybean yield and economics

It has been reported in the literature that soybean yields decrease following a pennycress crop (Johnson et al. 2017). Therefore, this model estimated the potential economic impacts of decreases in soybean yield. Table 5.3 summarizes the average soybean enterprise budget values over the 2008-2017 period for farms in these regions. During this timeframe, soybean production averaged 34.76 bushels per acre, with an average value of \$10.22 per bushel. Yield ranged from 29.59-44.31 bushels per acre. The lowest price during this period was \$8.37 per bushel while the highest price was \$13.92 per bushel. The average gross income per acre totaled \$371.41 with a range of \$291.05-517.45. Average direct expenses totaled \$245.95 per acre with a range of \$204.86-273.36. Average overhead expenses totaled 53.60 per acre with a range of \$40.18-60.65. Average net return per acre ranged from -\$6.19-210.31, with an average of \$71.86.

Table 5.4 summarizes the impact of potential soybean yield losses. We estimated yield losses ranging from 5%-30%. A 5% loss of soybean yield reduces net return to \$54.21 per acre compared to the 10-year average return of \$71.86. In the 10% yield loss scenario, net return is reduced to \$36.45 per acre. A 15% loss results in a net return of \$18.69 per acre, while a 20% loss results in a net return of \$0.93 per acre. The 25% yield loss scenario results in a net loss of \$16.83 per acre and the 30% loss results in a net loss of \$34.60 per acre. Notably, at the 10-year average soybean price of \$10.22 per bushel and the 10-year average combined direct and overhead expenses of \$299.55 per acre, soybeans must achieve a yield of 27.72 bushels per acre for the producer to break even under the current assumptions for crop insurance and other income per acre. This is an approximately 20.27% reduction in yield.

5.3.4 Potential scenarios for the corn-pennycress-soybean rotation

Since pennycress production will occur within a corn and soybean rotation, we estimated potential net returns of the three-crop rotation under different scenarios. Table 5.5 summarizes the average corn enterprise budget. During this timeframe, corn production averaged 134.24 bushels per acre with an average value of \$3.97 per bushel. Yield ranged from 113.14-166.95 bushels per acre, and price ranged from \$3.03-\$6.47 per bushel. Average gross return totaled \$556.79 per acre and ranged from \$400.85-823.42 per acre. Average direct expenses totaled \$418.86 and ranged from \$347.61-\$471.38 per acre. Average overhead expenses totaled \$87.41 per acre and ranged from \$61.15-\$98.70. Net return per acre averaged \$50.52 per acre and ranged from -\$68.30-310.38 per acre.

To understand the potential economic impact of the three-crop system, we generated different scenarios by adding estimated net returns of corn, pennycress, and soybean. Scenarios included pennycress net return at three different yield levels. Soybean net return included the 2008-2017 average and net returns from yield losses ranging from 5%-30%. In each scenario, corn net return was held constant at the 2008-2017 average of \$50.52 per acre. The estimated combined net returns of the three-crop system are shown in Table 5.6. In this model, the net return of the highest yielding pennycress paired with the 2008-2017 average soybean net return and the 2008-2017 average corn net return totaled \$220.12. This scenario produced the largest return. The net return of the lowest yielding pennycress paired with the net return of the 30% yield loss soybean and the 10-year average corn net return produced a net loss of \$136.98. This was the lowest producing scenario. Notably, the lowest yielding pennycress scenario, paired with any soybean scenario and the average corn scenario, never produces a positive net return. The middle yielding pennycress produces a positive net return when paired with the 2008-2017 average soybean and the 2008-2017 average corn, and the 5% yield-loss soybean and the 2008-2017 average corn. Conversely, the highest yielding pennycress produces a positive net return in every soybean yield scenario when paired with the 10-year average corn return.

5.4 Discussion

The breakeven yield is an estimate of the yield required for gross return to equal total expenses -i.e. a net return of \$0.00 – at a given price point. Our model estimates total direct and overhead production expenses of \$287.44 per acre. Based on the 2008-2017 average canola price of \$19.22 per cwt, our model predicts a breakeven pennycress yield

of 1,495 pounds per acre. To our knowledge, this is the first time a breakeven yield has been reported for pennycress. However, a personal communication by J. Sedbrook that appeared in a review by Cubins et al. noted that yields need to reach 1684 kg/ha to offset production costs (Cubins et al., 2019). A yield of 1684 kg/ha translates to 1502.43 pounds/acre, which is similar to our prediction.

Our model predicts a wide range of net return based on yield. Using yields reported in the literature, we estimated return for low (*Pennycress*, 2018), middle, and upper (Sedbrook et al., 2014) level yield scenarios using the 2008-2017 average price received by farmers, \$19.22 per cwt and production expenses of \$287.44 (Table 5.2). In the lowest yielding scenario, pennycress production potentially loses \$152.90 per acre at a price of \$19.22 per cwt and a yield of 700 pounds per acre. Pennycress production potentially loses \$99.13 per acre in the middle-yielding scenario of 979.76 lbs. per acre. The net return of the highest yielding scenario potentially generates \$97.74 at a yield of 2004.05 pounds per acre (2250 kg per ha). In contrast, the average canola yield for the North Dakota and Minnesota production regions is 16.99 cwt per acre during this period. The average net return for canola is \$52.90 per acre.

Pennycress production occurs as part of a system, and this system may experience economic impacts resulting from incorporating a new crop. For example, some studies report that pennycress influences soybean yield (Ott et al., 2019), potentially resulting in a yield loss. Therefore, our model estimated potential economic impacts of soybean yield reductions. Using production data from the same region as our canola-based pennycress

estimates, we calculated different scenarios of soybean yield loss, summarized in Table 5.4. The 10-year average net return of soybean production totaled \$71.86 per acre. Assuming pennycress reduces soybean yields by 5%, we found that net return from soybeans dropped to \$54.23. A 10% yield reduction resulted in a loss of \$36.72 per acre while a 15% yield reduction resulted in a loss of \$18.71 per acre. At a 20% soybean yield reduction, net return drops to \$0.94 per acre. The 25% yield loss scenario results in a net loss of \$16.81 per acre and the 30% loss results in a net loss of \$34.58 per acre. Clearly, potential soybean yield impacts are critical to the system. Based on the 2008-2017 average soybean price of \$10.22 per bushel and the 2008-2017 average combined direct and overhead expenses of \$299.55 per acre, soybeans must achieve a yield of 27.72 bushels per acre for the producer to break even under the current assumptions for crop insurance and other income per acre. Thus, an approximately 20.27% reduction in yield from the 2008-2017 average. Producers may be skeptical about adopting a new crop if it cuts into the bottom line of crops they are currently producing. The soybean yield impacts are critical considerations for pennycress adoption.

Since corn production is another critical component to this system, we estimated the total net return of the corn-pennycress-soybean rotation. Using production data from the same region as our canola-based pennycress estimates and soybean estimates, we calculated 2008-2017 average corn return, which was \$50.52 per acre. Then, we generated different production scenarios to estimate total net return of the corn-pennycress-soybean system. The lowest yielding pennycress paired with any soybean scenario and the 2008-2017 average corn return always generates a net loss. The middle yielding pennycress paired

with the 10-year average soybean return or the 5% yield loss and along with the 2008-2017 average corn return generates a positive net return. The highest yielding pennycress scenario generates a positive net return when paired with any soybean scenario along with the 2008-2017 average corn return. There is a wide range in each scenario, and this indicates that pennycress yield and soybean yield are highly critical to net return.

Our model makes several assumptions that have a potentially large impact on economic outcomes. The expenses included in this model, while in the range of those reported in the literature for pennycress (Julija A. Cubins et al., 2019), may differ from a real-world production scenario. For example, our model estimated seed costs at \$49.99 per acre based on ten-year average of canola seed expenses in North Dakota and Minnesota. In contrast, seed costs for common cover crops like oats, wheat, or rye total around \$5-10 per acre, according to SARE (Myers et al., 2019). Meanwhile, more expensive cover crop mixes that include legumes cost up to \$50 per acre. Additionally, the price received by farmers, \$19.22, may differ in a real-world production scenario. Given that pennycress is not yet a well-recognized crop and currently lacks a clear market, the price received by farmers may be lower. On the other hand, given the potential high-value bio-based products, like biofuel, lubricants, or plastics, pennycress may demand a higher price compared to canola.

Cover crops provide ecosystems services, which may improve profitability of the crop system. Our model does not include potential economic benefits of ecosystem services. For example, planting cover crops may improve soil fertility, suppress weeds and pests

(Dabney et al., 2001; Schipanski et al., 2014). As a result, producers may reduce fertilizer, herbicide, and pesticide applications, which could reduce input costs. It may take several years of planting cover crops before these savings come to fruition. These factors should be explored in future economic analyses.

5.5 Conclusion

Based on these estimates, we conclude that pennycress yield is a critical factor in the profitability of pennycress as a single crop and as part of the three-crop system. Plant breeders should strive for a minimum yield of 1,495 pounds per acre for producers to break even. Potential soybean yield reductions are also critical to the profitability of the system. Plant breeders and agronomists should strive to minimize soybean yield reductions in the three-crop system.

More work is needed to assess on-farm production economics of pennycress, as well as the potential impacts of the three-crop rotation. Our model used production data for a different crop, canola, and actual values for pennycress production will likely vary. For example, actual expenses of pennycress production may vary substantially from production expenses of canola. Additionally, canola price may not apply to pennycress. Given pennycress is not a traditionally recognized oil and protein source, it may fetch a lower price compared to canola. On the other hand, given its potential sustainability impacts, pennycress may fetch a premium price compared to other oilseeds. Finally, the protein content and quality as well as the oil profile may also influence price. For example, if pennycress has a higher, more bioavailable protein content it may demand a

higher price in the market. Additionally, if pennycress produces a more desirable oil profile, it may demand a higher price than other oils.

Future work should assess the potential economic benefits of ecosystem services provided by pennycress. Given cover crops have the potential to reduce soil compaction, sequester soil nutrients, reduce water pollution, and suppress weeds, there may be economic benefits not captured by our model. More work is needed to understand how these ecosystem services may contribute to the economic output of farm production. Having a better understanding of the economic impact of ecosystem services could influence producer adoption.

5.6 Relevance to the nutrition ecology framework and the food system

This project primarily aligned with the environmental and economic dimensions of the nutrition ecology framework. As previously discussed, pennycress development clearly aligns with the environmental dimension of the nutrition ecology framework given the potential environmental benefits of including pennycress in conventional crop rotations. The economic dimension encompasses industry, market, and other economic factors. This project estimated on-farm production economics of the pennycress system based on 10-year average data from canola, corn, and soybean producers in the Midwest. This study focused on farm-level production economics because this is the first step in the supply chain. Ensuring the economic viability of production is essential to ensure that sustainable, healthful foods can enter the market and reach consumers.

Tables and figures

Table 5.1 - Canola enterprise budget

	Average	Minimum	Maximum
Acres	310.03	247.21	398.88
Yield per acre (cwt.)	16.99	12.83	19.84
Operators share of yield %	100	100	100
Value per cwt.	19.22	15.87	28.43
Total product return per acre	326.56	282.58	416.95
Hedging gains/losses per acre	0	0.01	0.01
Crop insurance per acre	12.77	0.31	31.73
Other crop income per acre	1	0.19	3.81
Gross return per acre	340.34	294.87	429.07
Direct Expenses			
Seed	49.99	31.34	62.04
Fertilizer	64.88	51.03	78.36
Crop chemicals	26.02	22.21	31.18
Crop insurance	15.31	12.93	22.23
Fuel & oil	15.93	9.9	21.82
Repairs	19.44	15.66	24.98
Custom hire	5.07	3.06	7.2
Hired labor	0.1	0.05	0.53
Land rent	43.6	36.1	51.19
Operating interest	3.96	3.03	4.86
Miscellaneous	0.52	0.11	0.87
Total direct expenses per acre	244.81	210.73	284.55
Return over direct exp per acre	95.52	26.15	144.52
Overhead Expenses			
Custom hire	1.01	0.01	2.53
Hired labor	5.41	4.04	6.92
Machinery leases	3.62	2.79	6.78
Building leases	0.42	0.15	1.01
Farm insurance	3.25	2.35	4.47
Utilities	2.3	1.73	3.38
Dues & professional fees	0.66	0.38	0.94
Interest	3.24	2.5	3.68

Mach & bldg depreciation	20.05	11.7	33.13
Miscellaneous	2.66	2.09	3.25
Total overhead expenses per acre	42.63	29.04	55.92
Total dir & ovhd expenses per acre	287.44	242.25	330.53
Net return per acre	52.9	-29.77	101.95

Report Number 468002

FINBIN (2019). Center for Farm Financial Management: University of Minnesota.
Retrieved from <http://finbin.umn.edu> (originally created February 26, 2019).

Table 5.2 - Pennycress enterprise budget with different yield scenarios

	Low yielding	Middle yielding	High yielding
Yield per acre (cwt.)	7	9.79	20.04
Operators share of yield %	100	100	100
Value per cwt.	19.22	19.22	19.22
Total product return per acre	134.54	188.31	385.18
Hedging gains/losses per acre	0	0	0
Crop insurance per acre	0	0	0
Other crop income per acre	0	0	0
Gross return per acre	134.54	188.31	385.18
Direct Expenses			
Seed	49.99	49.99	49.99
Fertilizer	64.88	64.88	64.88
Crop chemicals	26.02	26.02	26.02
Crop insurance	15.31	15.31	15.31
Fuel & oil	15.93	15.93	15.93
Repairs	19.44	19.44	19.44
Custom hire	5.07	5.07	5.07
Hired labor	0.1	0.1	0.1
Land rent	43.6	43.6	43.6
Operating interest	3.96	3.96	3.96
Miscellaneous	0.52	0.52	0.52
Total direct expenses per acre	244.81	244.81	244.81
Return over direct exp per acre	-110.27	-56.50	140.37
Overhead Expenses			
Custom hire	1.01	1.01	1.01
Hired labor	5.41	5.41	5.41
Machinery leases	3.62	3.62	3.62
Building leases	0.42	0.42	0.42
Farm insurance	3.25	3.25	3.25
Utilities	2.3	2.3	2.3
Dues & professional fees	0.66	0.66	0.66
Interest	3.24	3.24	3.24
Mach & bldg depreciation	20.05	20.05	20.05
Miscellaneous	2.66	2.66	2.66

Total overhead expenses per acre	42.63	42.63	42.63
Total dir & ovhd expenses per acre	287.44	287.44	287.44
Net return per acre	-152.9	-99.13	97.74

Low yielding yield per acre adapted from:

Pennycress. (2018). Agriculture Marketing Resource Center.

<https://www.agmrc.org/commodities-products/grains-oilseeds/pennycress>.

Middle and high yielding yield per acre adapted from:

Sedbrook, J. C., Phippen, W. B., & Marks, M. D. (2014). New approaches to facilitate rapid domestication of a wild plant to an oilseed crop: Example pennycress (*Thlaspi arvense* L.). *Plant Science*, 227, 122–132.
<https://doi.org/10.1016/j.plantsci.2014.07.008>

Other values adapted from canola enterprise budget: FINBIN Report Number 468002
 FINBIN (2019). Center for Farm Financial Management: University of Minnesota.
 Retrieved from <http://finbin.umn.edu> (originally created February 26, 2019).

Table 5.3 - Soybean enterprise budget

	Average	Min	Max
Acres	495.71	438.51	554.61
Yield per acre (bu.)	34.76	29.59	44.31
Operators share of yield %	100		
Value per bu.	10.22	8.37	13.92
Other product return per acre	0.01	0	0.02
Total product return per acre	355.12	279.01	510.4
Hedging gains/losses per acre	0.44	-0.49	1.55
Crop insurance per acre	14.2	4.9	41.02
Other crop income per acre	1.64	0.56	3.05
Gross return per acre	371.41	291.05	517.45
Direct Expenses			
Seed	59.47	40.46	68.8
Fertilizer	18.96	12.62	26.72
Crop chemicals	22.78	15.22	30.1
Crop insurance	17.95	14.49	24.4
Drying expense	0.19	0.02	0.99
Fuel & oil	16.02	10.89	20.11
Repairs	21.45	18.9	23.87
Custom hire	4.48	2.8	5.65
Hired labor	0.82	0.3	2.04
Land rent	76.48	57.41	88.09
Machinery leases	1.21	0.41	2.34
Operating interest	4.49	3.65	5.39
Miscellaneous	1.64	0.43	2.46
Total direct expenses per acre	245.95	204.86	273.36
Return over direct exp per acre	125.46	53.7	262.72
Overhead Expenses			
Custom hire	0.72	0.15	2.09
Hired labor	8.33	5.95	9.22
Machinery leases	3	2.33	3.52
Building leases	0.67	0.45	0.93
Farm insurance	4.58	3.14	5.06
Utilities	3.08	2.4	3.68
Dues & professional fees	2	1.76	2.3
Interest	3.62	3.36	3.97
Mach & bldg depreciation	24.15	14.85	29.43

Miscellaneous	3.44	3.02	4.1
Total overhead expenses per acre	53.6	40.18	60.65
Total dir & ovhd expenses per acre	299.55	248.98	334.01
Net return per acre	71.86	-6.19	210.31

Report number 468969

FINBIN (2019). Center for Farm Financial Management: University of Minnesota.

Retrieved from <http://finbin.umn.edu> (originally created March 1, 2019).

Table 5.4 - Soybean yield loss scenarios

	5% yield loss	10% yield loss	15% yield loss	20% yield loss	25% yield loss	30% yield loss
Yield (bu/acre)	33.022	31.284	29.546	27.808	26.07	24.332
Average price (\$/bu)	10.22	10.22	10.22	10.22	10.22	10.22
Average insurance, hedging/gains and other income per acre	16.28	16.28	16.28	16.28	16.28	16.28
Gross return	353.76	336.00	318.24	300.48	282.72	264.95304
Average total Direct and overhead expenses	299.55	299.55	299.55	299.55	299.55	299.55
Net return	54.21	36.45	18.69	0.93	-16.83	-34.60

Values calculated and adapted based on FINBIN enterprise budget: Report number 468969

FINBIN (2019). Center for Farm Financial Management: University of Minnesota.

Retrieved from <http://finbin.umn.edu> (originally created March 1, 2019).

Table 5.5 - Corn enterprise budget

	Average	Min	max
Acres	289.19	247.58	327.7
Yield per acre (bu.)	134.24	113.14	166.95
Operators share of yield %	100	100	100
Value per bu.	3.97	3.03	6.47
Other product return per acre	0.79	0.03	1.61
Total product return per acre	533.26	392.92	815.95
Hedging gains/losses per acre	1.87	-0.69	3.92
Crop insurance per acre	19.52	4.07	55.54
LDP income per acre	0	0.01	0.01
Other crop income per acre	2.14	0.73	4.34
Gross return per acre	556.79	400.85	823.42
Direct Expenses			
Seed	83.88	58.28	92.06
Fertilizer	110.2	85.03	134.78
Crop chemicals	23.66	18.42	26.31
Crop insurance	21.46	17.83	29.11
Drying expense	9.29	1.94	25
Storage	0.42	0.08	0.96
Fuel & oil	26.83	17.35	34.21
Repairs	36.41	29.1	41.8
Custom hire	7.28	3.99	9.14
Hired labor	1.47	0.84	3.28
Land rent	84.94	55.74	106.65
Machinery leases	1.63	0.47	2.63
Hauling and trucking	0.99	0.24	1.52
Marketing	0.49	0.11	0.8
Operating interest	8.13	6.97	10.04
Miscellaneous	1.78	1.09	2.34
Total direct expenses per acre	418.86	347.61	471.38
Return over direct exp per acre	137.93	30.39	393.38
Overhead Expenses			
Custom hire	1.14	0.18	5.29
Hired labor	15.22	11.34	17.91
Machinery leases	5.65	4.01	7.53

Building leases	1.15	0.57	2.26
Farm insurance	5.9	3.55	6.93
Utilities	4.19	2.83	4.91
Dues & professional fees	2.61	2.02	3.34
Interest	6.14	5.43	7.2
Mach & bldg depreciation	40.44	23.93	48.96
Miscellaneous	4.99	3.98	5.95
Total overhead expenses per acre	87.41	61.15	98.7
Total dir & ovhd expenses per acre	506.27	412.76	563.49
Net return per acre	50.52	-68.3	310.38

Report Number 468913

FINBIN (2019). Center for Farm Financial Management: University of Minnesota.

Retrieved from <http://finbin.umn.edu> (originally created March 1, 2019).

Table 5.6 - Combined net return scenarios of the three-crop rotation

	Low yielding pennycress	Middle yielding pennycress	High yielding pennycress
Net return	-152.9	-99.13	97.74
10-year average soybean return	71.86	71.86	71.86
5% yield loss soybean return	54.21	54.21	54.21
10% yield loss soybean return	36.45	36.45	36.45
15% yield loss soybean return	18.69	18.69	18.69
20% yield loss soybean return	0.93	0.93	0.93
25% yield loss soybean return	-16.83	-16.83	-16.83
30% yield loss soybean return	-34.60	-34.60	-34.60
10-year average corn return	50.52	50.52	50.52
Range of returns	-136.98, -30.52	-83.21, 23.25	113.66, 220.12

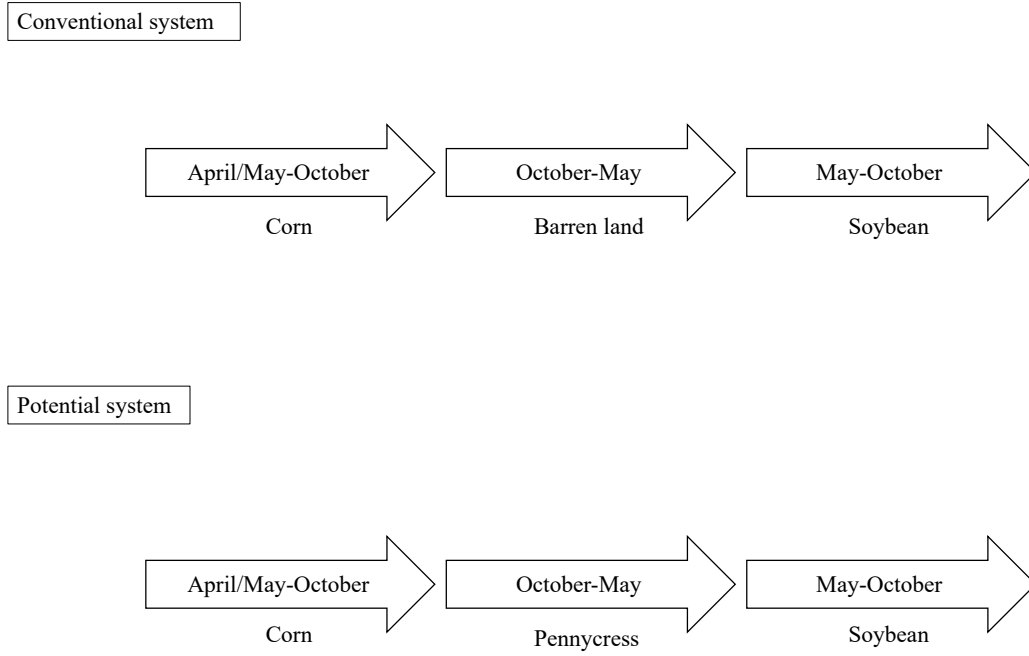


Figure 5.1 - Corn-pennycress-soybean system

Chapter 6: Intersection of diet, health, and environment: land grant universities' role in creating platforms for sustainable food systems

Abstract

Food and agriculture professionals strive to feed the growing population diets that maintain human health and minimize environmental impacts. Recently, global organizations such as the World Health Organization and the Food and Agricultural Organization have set goals related to the sustainable production of nutritious foods. In this vein, the EAT-Lancet Commission on Food, Planet, and Health has set dietary recommendations for human and planetary health. These recommendations, based on a wide body of evidence, suggest that plant-based diets are essential for the persistence of the population and the environment. This article briefly reviews the influence of diets on health and environmental outcomes in the context of the EAT-Lancet recommendations. Then the brief discusses the role of land grant universities in developing nutrition sensitive, environmentally resilient food systems. Then the article discusses local food system initiatives in Minnesota and offers insight into how the University of Minnesota may play a role in developing nutrition sensitive value chains by coordinating and supporting local food efforts. Ultimately, this brief suggests that land grant universities can help local communities equitably collaborate with upstream and downstream value chain actors to develop nutrition sensitive, environmentally resilient value chains.

An adapted version of this manuscript appeared in the journal *Frontiers in Sustainable Food Systems*. Len Marquart contributed to that manuscript.

6.1 Introduction

Food system professionals must feed a growing global population healthful, environmentally resilient diets (M. Clark et al., 2018). The global food system contributes to the triple burden of hunger, micronutrient deficiency, and obesity (Gomez & Barrett, 2013). Meanwhile, poor food production practices contribute to environmental degradation through greenhouse gas emissions, soil erosion, and decreased biodiversity. The negative implications of this system may exacerbate as the population surpasses 9 billion by 2050, and food production increases to meet demand (Tilman et al., 2011). Recognizing this wicked challenge, the World Health Organization (WHO) and the Food and Agriculture Organization (FAO) developed 17 cross-cutting Sustainable Development Goals that promote human and environmental health (FAO, 2017a). These goals provide orientation and direction for local food systems to achieve diets that support human and environmental health.

Local food systems are increasingly important as consumers demand transparent, equitable food systems ((Nel) Wognum et al., 2011). Many efforts strive to improve agricultural sustainability; provide equitable, culturally appropriate access to safe, nutritional foods; and enhance economies. Despite inextricable linkages, these efforts often occur in isolation. Enhanced coordination of local efforts focusing on agriculture, nutritional food security, and economic development could conceivably yield major impacts on human health, environmental sustainability, and economic prosperity. Land grant universities provide a platform for enhanced coordination of these efforts while operating at the nexus of the public and private sectors (Korczyk et al., 2015). These

universities were established to provide a practical education of agricultural, mechanical, and classical studies to the public using federal land (Association of Public and Land-Grant Universities [APLU], 2012). Faculty, students, research platforms, agriculture experiment stations, and extension services (e.g. community outreach) are key components of this mission (Association of Public and Land-Grant Universities [APLU], 2012). Universities play a crucial role in generating research and engaging in outreach activities that assist local efforts to improve agricultural sustainability and to provide equitable access to healthful foods.

Here, we briefly discuss links between diets and environment by highlighting information published by the EAT-Lancet Commission on Food, Planet, and Health (EAT-Lancet) to provide a global context. We discuss the potential role of land grant universities in supporting the development of nutrition sensitive food systems. The article then describes Minnesota-based initiatives providing healthful, affordable, safe foods for communities as an example of local efforts linking community, university, and industry activities. Finally, the article recommends that the University of Minnesota can play a role in developing nutrition sensitive value chains.

6.2 Diet, health, and environment: global context and recommendations

Sustainable diets promote health and nutrition, minimize environmental impacts, remain economically feasible, and promote sociocultural wellness (Drewnowski, 2018).

Healthful diets include adequate amounts of protein-rich foods, fruits, vegetables, and fats. Dietary recommendations vary depending on demographic characteristics. However, dietary guidance, such as the Dietary Guidelines for Americans (DGAs), is based on

mean population requirements (US Department of Agriculture and US Department of Health and Human Services, 2015). The DGA-recommended dietary pattern should support the health of the general population, but achieving dietary guidance is difficult as the food supply is largely non-compliant with DGAs (Krebs-Smith et al., 2010; P. E. Miller et al., 2015). Dietary guidance has not traditionally accounted for the impact of diets on the environment, which presents an opportunity as we approach 2050.

A growing body of literature highlights the link between plant-based diets, nutrition, and environmental health. The EAT-Lancet report suggests diets promoting nutrition and physical health as well as environmental sustainability are essential for the persistence of the global population (Willett et al., 2019). Plant-based diets are associated with decreased mortality and decreased risk for chronic disease (Orlich et al., 2013; Satija et al., 2016, 2017). Increased vegetable and fruit consumption is associated with decreased risk for chronic diseases (Hung et al., 2004), including Type 2 Diabetes mellitus (T2DM) (Muraki et al., 2013), cardiovascular disease (CVD), cancer (Dagfinn Aune et al., 2017), and weight gain (Bertoia et al., 2015). Diets rich in whole grains are associated with decreased risk for T2DM, CVD, and cancer (Dagfinn Aune et al., 2016a; Parker et al., 2013). Similarly, nut and legume consumption is associated with decreased risks of CVD, T2DM, and all-cause mortality (Afshin et al., 2014; Dagfinn Aune et al., 2016b; Bernstein et al., 2010). This contrasts with diets high in animal-based foods, particularly red processed meats, which are associated with increased risk for chronic disease (G.-C. Chen et al., 2013; Feskens et al., 2013; Pan et al., 2012). Other studies indicate that plant-based diets have a reduced carbon footprint compared to diets high in animal-derived

foods. A 2017 analysis showed that consumption and production of fruits, vegetables, and grains have lower impacts on global warming via decreased greenhouse gas emissions compared to red meat (Clune et al., 2017). This supports a 2017 study assessing environmental impacts of agricultural production systems and food choice, which showed that animal-derived foods have a negative environmental impact compared to plant-based foods (M. Clark & Tilman, 2017). Other reports show clear links between dietary patterns and environmental impacts, with plant-based diets having considerably reduced impacts compared to those that include meat (Aleksandrowicz et al., 2016; Hallström et al., 2015; Nelson et al., 2016).

The parallel benefits of plant-based diets for human and environmental health may influence future dietary recommendations, as shown by the EAT-Lancet report (Willett et al., 2019). The report recommends a diet low in animal-derived foods like red meat and dairy and encourages increased vegetable, fruit, legume, and whole grain consumption (Willett et al., 2019). This is similar to the DASH and Mediterranean diets, which purportedly have lower environmental impacts compared to Western diets (Nelson et al., 2016). While this diet would likely improve human health and reduce greenhouse gas emissions, some question its one-size-fits-all nature. The WHO withdrew support due to questions around the feasibility and cultural suitability of the diet (*WHO Withdraws Endorsement of EAT-Lancet Diet*, 2019). The cultural significance of food and agriculture is largely absent from the report. Future iterations might benefit by incorporating inclusive, socio-cultural perspectives. Despite these limitations, EAT-

Lancet provides evidence-driven insight into the influence of sustainable diets and sets goals for food systems professionals.

Developing foods and diets that sustain planetary and human health requires multiple disciplinary, systems approaches that account for nutrition, food science, agriculture, ecology, economics, policy, and socio-cultural factors (Barbier & Burgess, 2017; Tu et al., 2019). Systems approaches are becoming critical in addressing complex societal issues like obesity (T. T. Huang et al., 2009) and conservation agriculture policy (Ribeiro et al., 2016). However, these approaches are often difficult to conceive and coordinate due to their large size and broad scope. Land grant universities could play a critical role in implementing and training the next generation of professionals to use these approaches.

6.3 The role of land grant universities in developing nutrition sensitive food systems

Land grant universities can play a leading role in using food systems frameworks to assess the agronomic, economic, public health, and sociocultural aspects of sustainable diets. For example, in cooperation with on-campus faculty and outreach centers, agricultural experiment stations can research the ecological implications of planting crops that support recommended dietary patterns. Concomitant applied economic studies can evaluate the economic feasibility of agricultural changes (Boland et al., 2016). From a community perspective, university-led studies might assess public health and socio-economic impacts of populations adapting to sustainable dietary guidance. University extension programs may assess cultural appropriateness of dietary recommendations (Hassel, 2014; Hassel et al., 2019). Overall, these data might help food systems actors

create comprehensive, holistic strategies to develop sustainable, healthful, economically feasible food systems.

There are barriers to enacting this type of approach. Despite the interlinked nature of our food system, academic research and training remains largely siloed. Research activities occur in a segmented, disciplinarily isolated manner, and institutional practices may directly inhibit the ability to cross disciplinary boundaries (Choi & Pak, 2007). Other institutional factors, like the tenure process and faculty recognition, are often predicated on single disciplinary approaches, which make it difficult to collaborate outside of a discipline (Weaver, 2008). Food systems approaches require researchers and partners to overcome this traditional paradigm, perhaps by structuring institutional processes to offer merit for multiple disciplinary systems approaches, particularly for young faculty.

Translating university research to practical application in community settings can be difficult. The complexity of health issues in diverse communities can hinder traditional research approaches (Graham et al., 2006; Minkler, 2005). These approaches are often limited by a lack of consideration for diverse sociocultural perspectives, which can reduce community acceptance (Hassel, 2014; Hassel et al., 2019). Community-based participatory action research (CBPAR), which integrates community stakeholders into project design and execution, may improve translation of research to practical application (N. Wallerstein & Duran, 2010). These approaches are reciprocal in nature and allow communities to take agency in driving research initiatives, which may improve outcomes compared to traditional interventions.

Other sectors adapting systems models may provide examples for the food sector. For instance, like the food system, healthcare is non-linear, dynamic, and unpredictable (Lipsitz, 2012). As a result, the medical sector has implemented systems models for research and training (Choi & Pak, 2006, 2007; Weaver, 2008). Some models emphasize multiple-disciplinary training and research, systems thinking, cross-cultural engagement, and professional skill (e.g. communication, collaboration, critical thinking, leadership, etc.) development (Sehgal et al., 2008; Stroud et al., 2017). The medical sector has also implemented CBPAR approaches to address health disparities in at-risk communities (B. Campbell, 2010; N. B. Wallerstein & Duran, 2006). Some STEM fields also integrate systems thinking into training programs (Fowler et al., 2019). Training the next generation in these approaches may improve land grant universities' impacts on food systems. Despite these challenges and limitations, many universities are pursuing integrative research and training approaches, with some highlighting the need for a future workforce of food systems analysts (Ingram et al., 2020). Therefore, it is important to contextualize and describe local food systems efforts. The University has a potential role to engage with local groups to develop more nutrition-sensitive food systems.

6.4 Local context - Minnesota food security: supply chain firms and influencers

Agriculture and food production are critical to the Minnesota economy. In 2019, Minnesota agricultural production was valued at over \$17 billion, making it the 5th largest agricultural state (Ye, 2019). Minnesota also hosts the headquarters of many multinational food companies, including General Mills, Cargill, Supervalu, Land O'Lakes, and Hormel (Table 6.1) (Minnesota Department of Employment and Economic

Development, 2020). The state also boasts many small to medium size food and beverage companies.

Despite a thriving agriculture and food sector, many Minnesotans remain food insecure. According to a 2016 study, Minnesota is 7th worst in the nation for access to healthful foods (Rausch et al., 2016). Food security impacts nearly 10% of Minnesota families due to many contributing factors including income, transportation, and difficulty to access benefits, like Supplemental Nutrition Assistance Program and the Women Infant and Children program (Minnesota Department of Health, 2019b, 2019a). Access to nutritional foods is largely tied to socioeconomic status. In 2017, the Center for Disease Control found that prevalence of obesity increased in US adults as income and education levels decreased (Ogden et al., 2017). This may be due to the decreased costs associated with energy dense, high sugar foods compared to nutrient-rich foods (Andrieu et al., 2006; Monsivais & Drewnowsky, 2010). Disproportionate access to high-calorie, low-nutrient-dense foods may contribute to prevalence of obesity in Minnesota. Over 60% of Minnesota adults are overweight, and 30% are obese, resulting in \$3 billion in healthcare expenditure (Minnesota Department of Health, 2019a).

Food supply chains determine critical factors that influence diet and food choice, including price, availability, convenience, and safety. Supply chains include all actors and processes from production to consumption. As raw materials flow through the chain, key steps add value to the product, which gives rise to the term “value chain” (Figure 6.1). While the traditional value chain concept focuses solely on economic value, there is

burgeoning interest in the concept of nutrition sensitive value chains (NSVCs) (FAO, 2017a, 2017b; *Identifying Opportunities for Nutrition-Sensitive Value-Chain Interventions* | IFPRI, n.d.; Uccello et al., 2017). These aim to enhance supply and demand for nutritious foods while sustainably adding nutritional value at critical chain stages (FAO, 2017b). This framework examines links between nutrition issues in target populations and constraints in supply and demand for foods that may address these problems (de la Peña & Garrett, 2018). Ultimately, NSVCs deployed at the local level improve availability, affordability, quality, and acceptability of nutritious food.

Many initiatives throughout Minnesota aim to improve access to healthful, sustainable, affordable foods, and these efforts could align with a NSVC framework. For example, the Minnesota Food Charter, developed by multi-disciplinary, multi-sector food leaders launched in 2014 to provide a roadmap to guide policymakers and community leaders in providing Minnesotans with access to safe, equitable, affordable, healthful foods (*What Is the Minnesota Food Charter?*, n.d.). The Charter also formed a network connecting key players working in food equity and sustainability like Second Harvest Heartland, the Good Acre, and the Regional Sustainable Development Partnerships (RSDP).

Second Harvest Heartland and The Good Acre improve access to healthful foods and strengthen local agriculture in the Twin Cities through community partnerships. Second Harvest Heartland recovers food throughout the supply chain, which it then distributes to food pantries and other community partners throughout Minneapolis and St. Paul (*About Us* | *Second Harvest Heartland*, n.d.). They also house a hydroponic farm that facilitates

hands-on participation in sustainable farming while supplying fresh produce year-round (*Our Farm | Second Harvest Heartland*, n.d.). The Good Acre, a food hub in Falcon Heights, connects farmers, producers, and communities throughout the local food system. They operate a wholesale program that provides schools with fresh produce and implement culinary and agriculture programs to ensure the integrity and safety of agricultural products (*The Good Acre Annual Report*, 2017).

Several initiatives connect and reconnect indigenous cultures to the food system. The Seeds of Native Health Campaign for Indigenous Nutrition, led by the Shakopee Mdewakanton Sioux Community, works to restore food sovereignty for Minnesota indigenous communities. The Shakopee Mdewakanton Sioux Community hosts an annual Conference on Native American Nutrition, which convenes tribal officials, researchers, practitioners and others to discuss indigenous and academic knowledge regarding Native health and food (“2019 Native Nutrition Conference,” 2019). Dream of Wild Health is another group reconnecting indigenous communities with their cultural roots through food via youth programs, CSAs, food demonstrations, a 10-acre farm, and a community garden (“Programs,” n.d.).

The RSDP is an Extension program aiming to improve agricultural sustainability while developing markets for local products (*RSDP Projects | UMN Extension*, n.d.). The group coordinates with programs like the Forever Green Initiative (FGI), which works to improve biodiversity and regenerative agriculture by incorporating new crops in conventional crop rotations (Forever Green, 2016). The RSDP also works with groups

facilitating entrepreneurial projects, like Grow North MN (*Resource Navigator Database*, n.d.). Each of these initiatives could be leveraged to develop NSVCs.

6.5 Nutrition sensitive value chains: an opportunity for improved health

Developing sustainable NSVCs could provide more Minnesotans with access to foods that support human and environmental health. NSVCs provide a framework to improve nutrition in Minnesota by connecting, coordinating, and supporting groups working on agriculture, food, and health. The University of Minnesota interfaces with upstream and downstream value chain actors as well as other value chain influences (Figure 6.2). Thus, it can play a key role in facilitating NSVCs.

The University of Minnesota houses many departments and research platforms that train the next generation of value chain actors. These departments can conduct research on individual parts of the value chain in a systemic and coordinated manner, as illustrated in Figure 6.3. For example, the Department of Agronomy and Plant Genetics plays a key role in improving crops and developing sustainable farming practices. The Department of Bioproducts and Bio Systems Engineering develops methods for processing raw materials into food ingredients. The Department of Food Science and Nutrition evaluates end-use, sensory, and nutritional qualities, and safety of raw materials and finished products. The Department of Applied Economics and the Carlson School of Business evaluate the production and supply chain economics. The School of Public Health can evaluate the broader health implications of production and consumption while the Institute on the Environment can assess sustainability impacts. Taken together, these

departments represent nearly the entire field-to-consumer continuum, and they can collaborate with local groups to develop NSVCs.

The University of Minnesota is well positioned to engage local groups working on individual parts of the value chain, which could improve food systems through enhanced coordination and collaboration. For example, RSDP, Grow North MN, and FGI develop new crop enterprises and cultivate local food entrepreneurship. These groups may benefit from coordinating with groups that work downstream in the value chain like Second Harvest and the Good Acre, who traditionally focus on supplying foods to citizens. Agricultural training offered through the Good Acre and Second Harvest may benefit from the networks and knowledge systems offered through FGI, RSDP, and Grow North MN. Bridging these groups clearly aligns with the mission of university extension programs by offering training opportunities to local communities. These relationships can advance mutual research goals for many value chain actors. Table 6.2 highlights potential collaborative relationships between value chain stakeholders and University of Minnesota Departments.

Given the industry and cooperative presence in Minnesota, there are ample opportunities to connect mid-stream actors (e.g. processing, storage, and logistics) to upstream (e.g. FGI, RSDP) and downstream (e.g. Good Acre, Second Harvest) entities, along with university departments (Table 6.2). Currently, few nutrition-focused efforts engage this tier of the value chain, yet it plays a major role in influencing consumption (Stuckler &

Nestle, 2012; Willett et al., 2019). Efforts occurring at this part of the value chain could provide an impactful new opportunity for university engagement.

Empowering community members to take leading roles in developing NSVCs is imperative to ensure key issues are prioritized and solutions are culturally appropriate.(Uccello et al., 2017) Creating safe environments for community groups (e.g. Dream of Wild Health) to equitably collaborate with other firms interested in developing NSVCs (e.g. FGI, RSDP, Good Acre, and Second Harvest) could yield unprecedented nutrition outcomes. These engagements could be brokered through CBPAR models, whereby community stakeholders identify critical health issues and collaborate with university and supply chain stakeholders to develop NSVCs that address these problems. Land grant universities offer a mechanism to align and unite across sectors and disciplines to sustainably develop NSVCs while fulfilling their land grant mission. This integrative approach can improve the relevancy and impact of the nutrition discipline while creating more sustainable, healthful, equitable, economically resilient food systems.

6.6 Conclusion

Food and agriculture professionals must provide the growing population healthful diets that support resilient food systems. Global initiatives provide goals and recommendations for more sustainable diets and food production (FAO, 2017a; Willett et al., 2019).

Developing sustainable NSVCs could achieve these goals (de la Peña & Garrett, 2018; FAO, 2017b; Uccello et al., 2017). Land grant universities can play a key role in assisting local efforts to develop NSVCs to ensure food security while fulfilling their mission of

research, outreach, and education. Actively participating in systems approaches is crucial for this process. The medical sector and STEM fields provide models for integrating systems approaches into research and training (Choi & Pak, 2006, 2007; Fowler et al., 2019; Stroud et al., 2017). Institutional processes that disincentivize multiple-disciplinary collaborations must be overcome to facilitate systems approaches. Perhaps adjusting these processes to emphasize more multiple disciplinary, systems-based work could stimulate food systems approaches and improve health and environmental outcomes. Creating processes for local communities to actively participate in the development of NSVCs is crucial. Building reciprocal relationships through CBPAR can help to integrate communities into this process. Given its strong ties to local food systems and global food producers along with its diverse institutional expertise, the University of Minnesota is well-positioned to play a key role in developing models and frameworks supporting the development of NSVCs. This integrative, systematic process can improve the efficacy of nutrition research by intimately engaging across the supply chain, thus effectively translating research into practical application. Coordinated, systems-approaches, rather than reductionist, single disciplinary approaches, are essential to address the complex challenges throughout 21st century food systems. Universities, industry, and policy makers can strive together to incentivize these systemic processes, through shared value and common purpose to ensure the persistence of human and planetary health.

6.7 Relevance to the nutrition ecology framework and the food system

The ideas presented in this chapter align with all four dimensions of the nutrition ecology framework. Land grant universities play a unique role in food system development. University faculty and staff foster research approaches that evaluate and assess food

system components. The university also interacts with industry, policy, community, and non-profit stakeholders throughout the food system. Coordinated engagements between these groups can influence the economic, environmental, social, and nutrition outcomes of the food system, and each of those components are critical components of the nutrition ecology framework. These relationships are highlighted in Figures 6.2 and 6.3, and specific examples of areas of collaboration are listed in table 6.2. Perhaps most importantly, the university trains the food systems work force. Taken together, the relationships and activities described throughout this chapter contribute to societal benefits through the development of sustainable food systems.

Tables and Figures

Table 6.1: Recommendation of value chain actors, influencers, and roles in Minnesota. This is a non-comprehensive overview.

Minnesota value chain actors, influencers, and roles	
Entity	Role
Farmers	Crop production
CHS	Agriculture, Co-operative
Cargill	Agriculture, processing
Land O'Lakes	Agriculture, processing
General Mills	Food processing
Hormel	Food processing
August Schell Brewing Company	Brewing
Surly Brewing	Brewing
Bay State Milling Company	Milling, ingredient manufacturing
Grain Millers	Milling, ingredient manufacturing
Calyxt	Crop development
Supervalu	Wholesaling and retailing
Target	Retailing
University of Minnesota College of Food, Agriculture, and Natural Resource Science	Training, research: agronomy, food science, nutrition, ecology
University of Minnesota School of Public Health	Training, research: nutrition and health epidemiology
University of Minnesota College of Veterinary Medicine	Training, research: food safety, animal health
University of Minnesota Medical School	Training, research: health
Regional Sustainable Development Partnerships	Extension, outreach
Forever Green Initiative	Crop development
Grow North MN	Agriculture and food entrepreneurship
Second Harvest Heartland	Food bank, aggregation, distribution
Good Acre	Food hub, community engagement: agriculture and culinary programs
Dream of Wild Health	Food sovereignty and Indigenous knowledge systems
Seeds of Native Health	Food sovereignty and Indigenous knowledge systems

Table 6.2: Recommendation of potential collaborations between Value chain actors and influencers, and the University of Minnesota. This is a non-comprehensive overview.

Minnesota value chain actors, influencers, and roles		
Entity	Role	Potential University of Minnesota collaborators
Farmers	Crop production	Department of Agronomy and Plant Genetics
CHS	Agriculture, Co-operative	Department of Agronomy and Plant Genetics
Cargill	Agriculture, processing	Department of Agronomy and Plant Genetics, Department of Bioproducts and Bio Systems Engineering, Department of Food Science and Nutrition
Land O'Lakes	Agriculture, processing	Department of Agronomy and Plant Genetics, Department of Bioproducts and Bio Systems Engineering, Department of Food Science and Nutrition
General Mills	Food processing	Department of Bioproducts and Bio Systems Engineering, Department of Food Science and Nutrition
Hormel	Food processing	Department of Food Science and Nutrition
August Schell Brewing Company	Brewing	Department of Food Science and Nutrition
Surly Brewing	Brewing	Department of Food Science and Nutrition
Bay State Milling Company	Milling, ingredient manufacturing	Department of Bioproducts and Bio Systems Engineering, Department of Food Science and Nutrition
Grain Millers	Milling, ingredient manufacturing	Department of Agronomy and Plant Genetics, Department

		of Bioproducts and Bio Systems Engineering, Department of Food Science and Nutrition
Calyxt	Crop development	Department of Agronomy and Plant Genetics, Department of Plant and Microbial Biology
Supervalu	Wholesaling and retailing	Department of Food Science and Nutrition, Department of Applied Economics
Target	Retailing	Department of Applied Economics
Grow North MN	Agriculture and food entrepreneurship	Department of Applied Economics, Department of Agronomy and Plant Genetics, Department of Bioproducts and Bio Systems Engineering, Department of Food Science and Nutrition
Second Harvest Heartland	Food bank, aggregation, distribution	Department of Applied Economics, Department of Food Science and Nutrition
Good Acre	Food hub, community engagement: agriculture and culinary programs	Department of Applied Economics, Department of Food Science and Nutrition, Department of Agronomy and Plant Genetics
Dream of Wild Health	Food sovereignty and Indigenous knowledge systems	Department of Applied Economics, Department of Food Science and Nutrition
Seeds of Native Health	Food sovereignty and Indigenous knowledge systems	Department of Applied Economics, Department of Food Science and Nutrition

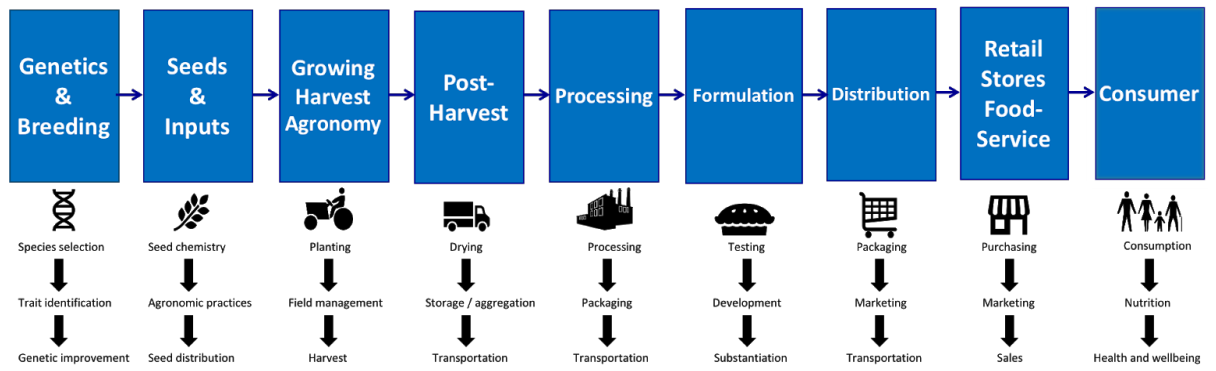


Figure 6.1 gives an overview of a hypothetical agricultural value chain.

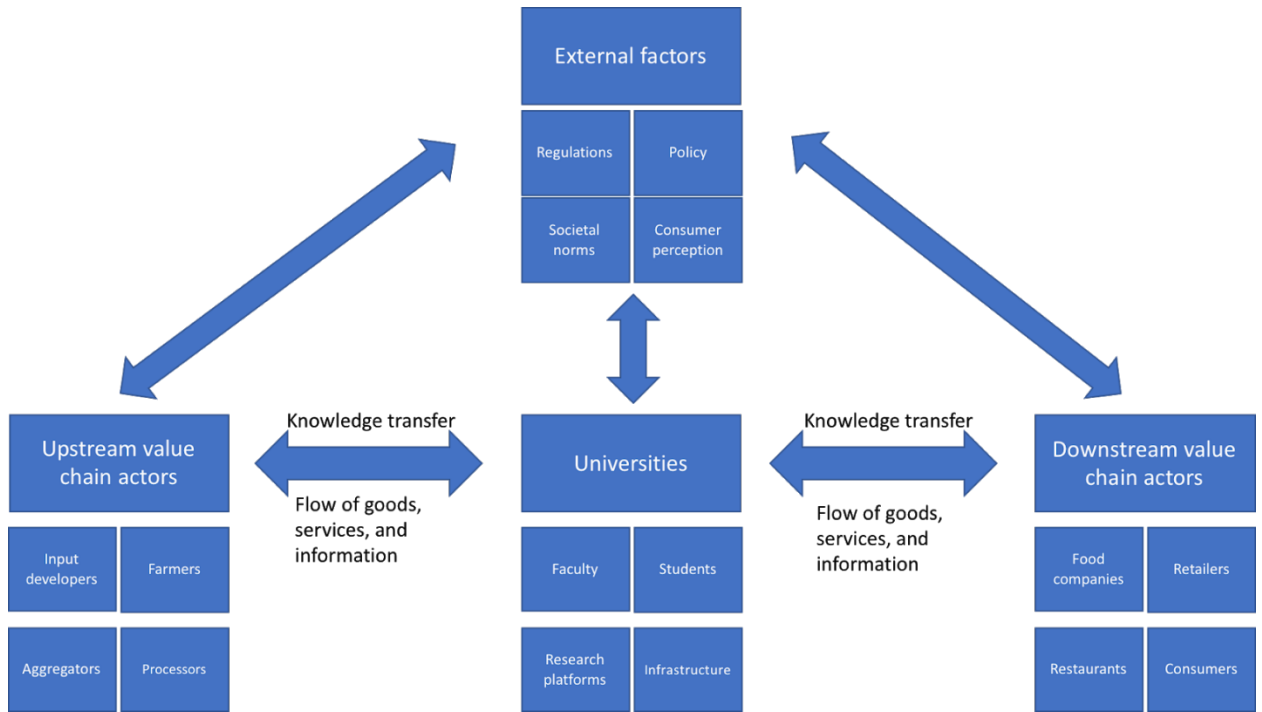


Figure 6.2 shows the reciprocal relationships between universities and value chain actors and influencers.

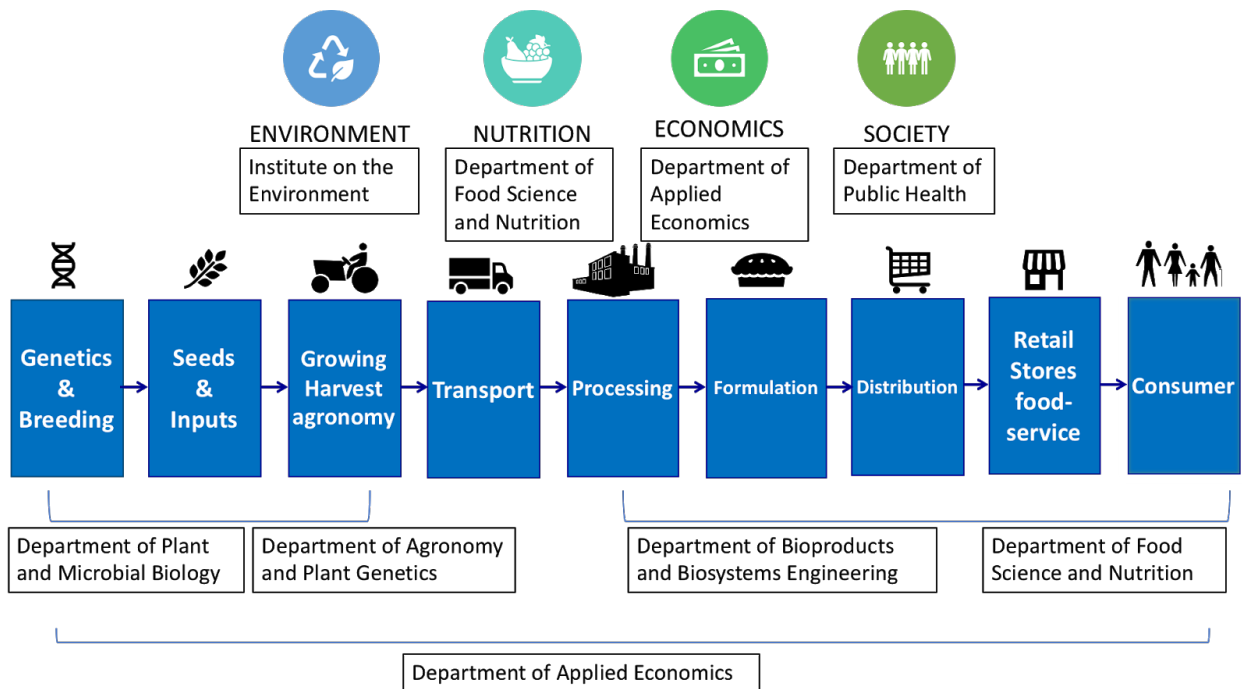


Figure 6.3 shows how several University of Minnesota departments could interact with the value chain.

Chapter 7: Critical reflections on a multi-disciplinary training framework for the fields of agriculture, food, and nutrition sciences

The field of nutrition is rapidly evolving to require systems approaches that address complex challenges throughout the food system. However, training approaches remain siloed. Experts within the field of nutrition agree that training programs ought to improve capacity in collaborating across disciplines and across sectors to foster systems approaches. There are few practical examples that integrate these concepts into graduate training programs. This paper describes a cross-disciplinary training program, rooted in experiential learning, that emphasized collaborations across diverse food systems components. Then the paper describes four domains of food systems learning. Examples of cross-disciplinary collaborations, learning outcomes, and key abilities for food systems professionals. Learning outcomes and key abilities are also described in the context of the T-shaped professional. Finally, key elements of food systems learning are described, and the program is placed in the context of the nutrition ecology framework.

An abstract based on this work, entitled “Moving Toward 2050: Supplementing Traditional Nutrition Programs with 21st Century Training,” appeared in the journal *Current Developments in Nutrition*, and it was presented to the American Society for Nutrition. Melissa Jansma, Therese Liffbrig, and Len Marquart appear as co-authors on the abstract.

7.1 Introduction

The fields of agriculture, food, and nutrition sciences must adapt training models to accommodate the complex, adaptive, cross-disciplinary nature of the food system. The complexity of the global food system has dramatically changed the role of food and nutrition professionals in the 21st century. Previously, nutrition scientists focused on the relationships between single nutrients, single foods, and single health outcomes, with little regard for systems implications (e.g. economic, environmental, sociocultural) outcomes of nutrition interventions (Ridgway et al., 2019). These linear approaches were largely successful in the early to mid 20th century and addressed health problems with one-to-one cause-and-effect relationships, like the micronutrient deficiency diseases goiter and pellagra (Leung et al., 2012; WHO, 2000). However, contemporary nutrition challenges produced by the increasingly complex global food system lack clear-cut, definitive solutions. For instance, managing the complex relationship between the food system, diet-related chronic disease, and environmental degradation has been described as the most pressing challenge of the 21st century (Barbier & Burgess, 2017; M. Clark et al., 2018; Drewnowski et al., 2020). Solving food systems challenges requires cross-disciplinary approaches accounting for nutrition, food science, agriculture, ecology, economics, policy, and socio-cultural factors (Barbier & Burgess, 2017; Tu et al., 2019). Implementing these approaches requires capacity building throughout the fields of agriculture, food, and nutrition sciences, whereby professionals are trained to understand the complex relationships and interlinkages throughout the food system, identify and prioritize development opportunities, and collaborate across sectors and disciplines to manage challenges.

Global leaders suggest that cross-disciplinary, cross-sector, systems-focused training is critical for agriculture, food, and nutrition scientists. Whereas previous training focused on solving single-disciplinary problems, 21st-century training must focus on managing complex, adaptive problems through systems approaches (Fardet & Rock, 2018). The High-Level Panel of Experts on Nutrition and Food Security (HLPE) described the need for capacity building across nutrition and food systems (HLPE, 2017). This involves training future professionals with a depth of disciplinary expertise and a breadth of experiences in cross-sector, cross disciplinary approaches. For example, nutrition professionals must understand key concepts in agricultural production, economics, public health, and social science. Meanwhile, Hickson and colleagues discuss critical needs to revise training requirements to include more hands-on learning and complementary skill development (Hickson et al., 2018). Further, Fanzo and colleagues describe critical professional skills for nutrition professionals in a post 2015 world, including communication, multi-sectoral collaboration, advocacy, leadership and technical skills (J. C. Fanzo et al., 2015). More broadly, several reports highlight the importance of systems-thinking in STEM fields, with many researchers developing training programs that explicitly teach these skills (Fowler et al., 2019). Similarly, Ingram and colleagues described the need for a workforce trained as food systems analysts to manage issues throughout the food system ranging from food security, environmental degradation, malnutrition, and economic disparities (Ingram et al., 2020). While experts across the food system in multiple disciplines have identified a need to implement cross-

disciplinary, cross-sector, systems-based education, there are few practical examples in the fields of nutrition and food science.

The ability to effectively work across scientific disciplines with an equal ability to communicate across fields of practice in the current work environment is essential for high functioning professionals to manage complex food and nutrition issues. This multifaceted approach are supported by the concept of the “T-shaped professional.” T-shaped professionals have a depth of technical knowledge complemented by a breadth of skills and experiences required to work across disciplines and sectors to manage complex, adaptive (i.e. wicked) problems. The vertical axis represents skills and disciplinary expertise, while the horizontal axis represents the professional skills and character attributes required to function effectively in the workforce. This concept is widely used in fields like business, engineering, information & technology, and conservation science (Conley et al., 2017; M. T. Hansen & von Oetinger, 2001; McIntosh & Taylor, 2013). Figure 7.1 illustrates the T-shaped professional concept and provides examples of complementary professional skills, abilities, and attributes, including communication, collaboration, critical thinking, cross-disciplinary engagement, cross-cultural engagement, systems thinking, leadership, management, problem solving, empathy, integrity, and accountability.

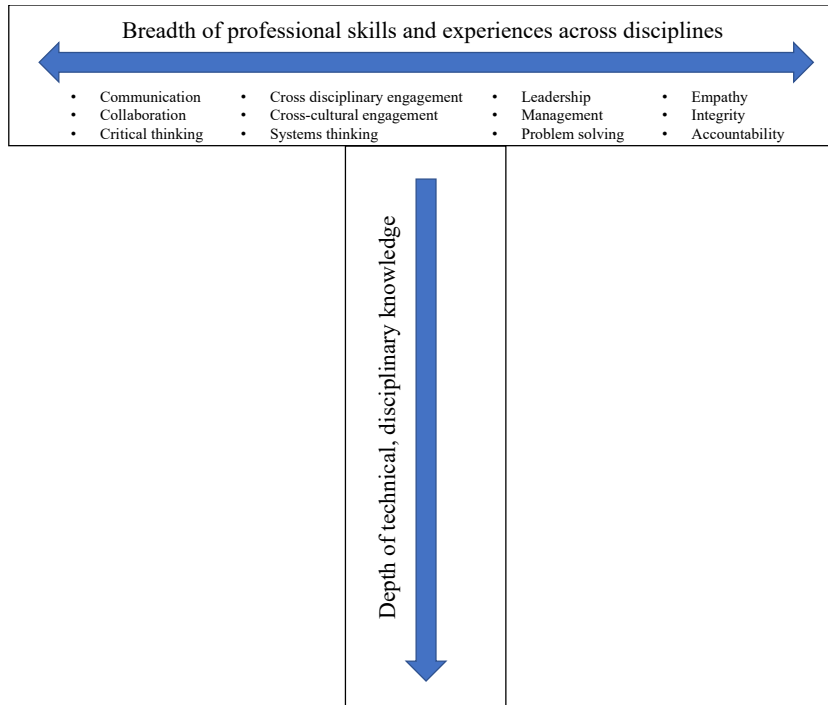


Figure 7.1 - The T-shaped professional model

Given the burgeoning interest in cross-disciplinary, cross-sector training and complementary skill development in the context of food systems, a research group at the University of Minnesota Department of Food Science and Nutrition aims to integrate these concepts into research and training programs. Here, we describe an individual case that was specifically designed to improve capacity for cross-disciplinary and cross-sector training, akin to the concept of the T-shaped professional. Emphasis was placed on understanding food systems linkages, developing cross-sector, cross-disciplinary abilities, and instilling key character attributes to develop T-shaped capabilities.

7.2 An interdisciplinary approach to training

The research and training that contributed to this dissertation occurred in a highly collaborative, interdisciplinary environment. Beyond the traditional curriculum and research required of a graduate student, the student intentionally participated in activities

and experiences outside the University system. These research and training experiences were meant to equip the student with abilities required to address complex, interconnected, 21st century food systems challenges. Activities and experiences were largely pursued based off the author's natural inclination along with guidance and insight provided by the academic advisor and graduate committee. Activities occurred in industry, academia, and the non-profit sectors. Collaborations occurred between disciplines throughout the supply chain, ranging from plant genetics to nutrition, public health, and regulatory science. Learning experiences were designed to expose the student to a wide range of food systems components to provide a holistic perspective and to instill key food systems abilities. While participating in the activities, the student assessed linkages and relationships between food systems components. This occurred largely through personal observation, reflection, journaling, and dynamic discussions between the author, his graduate advisor, food systems stakeholders, and academic committee members.

The following sections briefly describe four key domains that influence food systems and the disciplines engaged throughout each domain. Activities are categorized according to these domains to provide orientation for readers, educators, and food systems stakeholders. Domains were meant to capture diversity of perspectives, mindsets, and approaches throughout the food system, based on existing literature (Drewnowski et al., 2020; HLPE, 2017). Activities within each domain are described, and linkages between domains and activities are discussed. Then, key lessons from each domain are presented. Three food systems abilities are presented based on learnings from this program. The

final section describes critical reflections on experiences, including characteristics of the learning environment, student, and academic teams that are conducive for effective food systems learning. These reflections provide insight for future students pursuing interdisciplinary, multi-sector, food systems experiential learning.

7.3 Four domains for food systems learning

Broadly, the food system is comprised of all actors, activities, drivers, influencers, and outcomes involved in the production, delivery, and consumption of food (Drewnoski et al., 2020; HLPE, 2017). The food supply chain lies at the heart of this system, and it involves all processes from food production to consumption. This includes seed development (i.e. genetics and breeding), farm production, processing, retailing, and food service. The supply chain plays a major role in influencing food availability, pricing, quality, and environmental outcomes (Boland et al., 2016; (Nel) Wognum et al., 2011). Socio-political and economic drivers, like globalization; local, national, and international leadership; humanitarian crises, and market volatility, determine critical food systems outcomes. For instance, policies enacted at the local and national level greatly influence food access. Cultural drivers, like religion, rituals, social traditions, and socio-economic status, influence political, economic, and community processes which govern food system outcomes. For example, religious affiliation can influence dietary restrictions, and socio-economic status play a critical role in food access.

Clearly, the food system is complex and adaptive. Sectors and disciplines are intimately engaged and integrated throughout the food system. Changes occurring at one point in the system have downstream, often unintended, consequences that cascade throughout the

system. Historically, nutrition training, which has primarily focused on reductionist approaches, has not captured this complexity (Fardet & Rock, 2018; Scrinis, 2015c). We developed an integrated training program to allow the student to experience first-hand the intricacies and complexities throughout the food system. Here we present four key domains of food systems learning based on the author's research, training, and external learning activities. These four domains include: regulatory/policy, non-government/non-profit, research and development, and civic engagement/community. In this context, the policy/regulatory domain refers to the policies that enact regulations, which influence the food system as well as the food industry response to these regulations. The non-government/non-profit domain refers to activities conducted by external organizations (i.e. non-government, non-industry) working to influence the food system, through either policy opinions or research activities. The research and development domain includes the research activities, either occurring in industry, academia, or as a collaboration between the two, that contribute to the development of a food or crop. Finally, the civic engagement/community domain refers to the activities that advance public or community concerns. In this context, community refers to a group of people living in the same geographic location or sharing a common set of characteristics. Figure 7.2 illustrates these four components and highlights the nexus for food systems education.

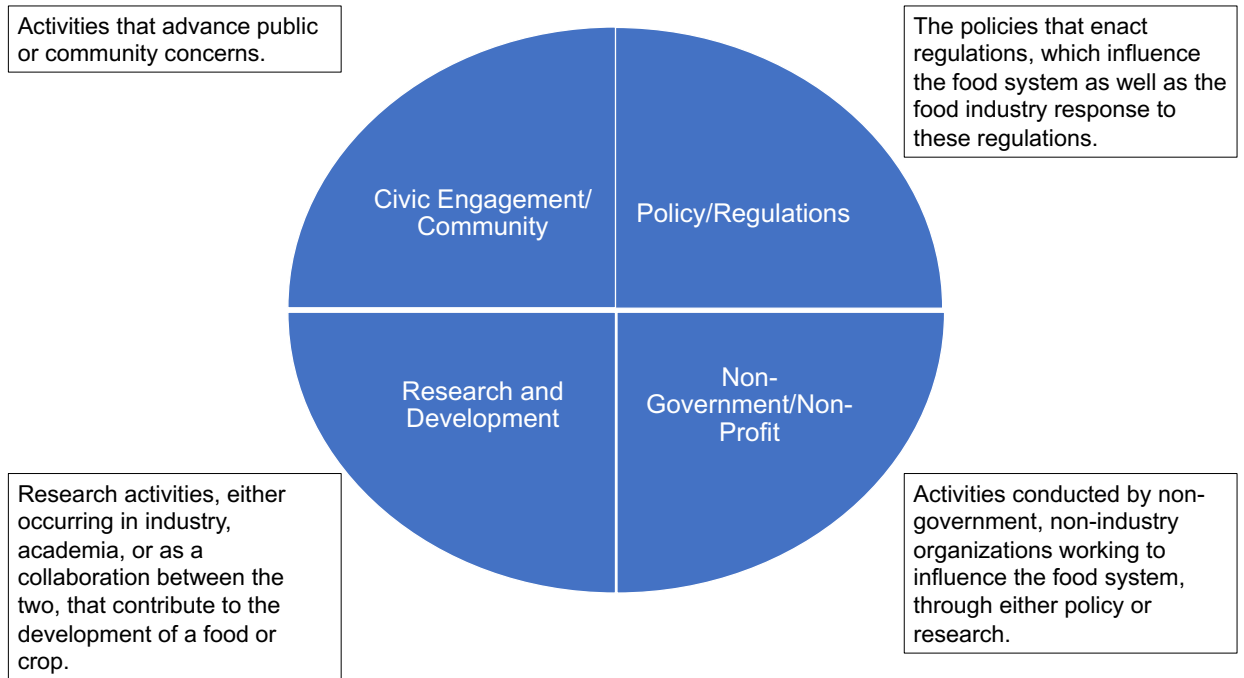


Figure 7.2 - Four domains of food systems learning

The following sections describe key activities and experiences within each learning domain. Sections highlight interconnections across food systems disciplines and show how disciplines interact with and influence other domains and activities. Key lessons from each domain are provided. After describing the activities and lessons within each domain, this section culminates in three abilities essential for food systems learning, which integrate concepts from each domain.

7.3.1 Domain 1: Policy/regulations

Domain 1 encompasses the intersection between the government and industry sectors, as policies enacted by the government regulate the food industry. The primary experience of Domain 1 is summarized in Table 7.1. The student worked in the corporate quality assurance department of a medium-sized milling and specialty ingredient company.

During this period, the United States Food and Drug Administration (FDA) enacted new policy requiring the food industry to change the nutrition facts panel (FDA, 2016b). The author collaborated across business units to develop a strategy to ensure that all products within the company portfolio complied with the regulations. During this time, the student also participated in other projects related to commercialization and raw material and supplier approval auditing processes.

Experiences in Domain 1 fostered T-shaped skills. The student applied disciplinary knowledge (e.g. nutrition science) in a practical context (e.g. labeling reform) while collaborating across disciplines. The student worked across multiple disciplines, including food science, nutrition, product development, food safety, processing, commercialization, and sales. These diverse disciplines are tightly integrated, and actions by a single discipline have consequences for other actors. Clear communication is essential for success. For example, communication between food scientists, product developers, and regulatory specialists is essential to ensure that products convey accurate, up-to-date nutrition information. This information plays a major role at fostering trust between the sales team and their customers. Experiences in this domain provided key insights into influence of policy on the food system, which provided a context and a basis for activities in other domains.

Table 7.1 – Domain 1: Policy/regulations

Domain 1: Policy/Regulation					
Location of experience	Role	Sectors engaged	Key Activity	Disciplines	Learning outcome
Food and ingredient company	Quality assurance associate	Industry, government	Regulatory compliance projects – 2016 NFP regulations, raw material & supplier auditing	Food science, nutrition, regulatory, processing, food safety, commercialization, sales	Understanding how regulations influence food industry processes – communication of nutrition information, safety of food ingredients, customer interactions

7.3.1.1 Linkages to other domains and activities

Learning experiences within the policy/regulatory domain provided useful insights for the non-government/non-profit domain, as well as the research and development domain.

Specifically, the experiences in the policy/regulatory domain allowed the author to intimately understand policy implications for the food industry, which was helpful as the non-government/non-profit domain experiences were largely based on influencing policy. Additionally, the policy/regulatory domain also provided insight into the processes required to prime new food ingredients for wider adoption, which was a critical component of the experiences within the research and development domain.

7.3.1.2 Key lesson

These projects provided a more holistic, systems-based perspective on the impact of regulations throughout a food business. This enhanced the authors ability to work across

disciplinary boundaries, which served as a basis for future projects in subsequent domains.

7.3.2 Domain 2: Non-government/non-profit

Domain 2 encompasses activities by non-government organizations (NGOs) and non-industry (i.e. non-profit organizations) groups that influence food systems actions. The primary experiences of Domain 2 are summarized in Table 7.2. First, the student worked with the Grains for Health Foundation to convene and collaborate with government, academic, industry, and other NGO partners to increase availability and access to whole grain foods. During this period, the student collaborated with a diverse, multi-disciplinary, cross-sector working group to better understand the impact of regulatory and supply chain issues on whole grain consumption. Regulatory uncertainty can decrease consumption of whole grain products. For instance, the lack of clarity around the definition of a whole grain food limits consumer understanding of whole grains and their benefits as part of a healthful diet. The supply chain also plays a major role in influencing whole grain consumption. For instance, the reduced shelf-life of whole grains compared to refined grains limits their appeal to chefs and product developers. Additionally, a lack of understanding of how to incorporate whole grains into recipes limits availability of whole grains on restaurant menus. In this case, the NGO worked to establish a research base aimed at reducing consumer confusion regarding whole grain definitions and mitigating supply chain issues limiting whole grain consumption.

The student also served as an adjunct fellow for the Institute on Science for Global Policy (ISGP). The ISGP convenes science and policy experts, spanning industry, government, and consumer advocacy groups, to discuss complex, emerging topics. The student participated in a weeklong session entitled Innovative Foods and Ingredients. During the session, stakeholders discussed and presented scientific evidence either in support, or against, emerging biotechnology-related foods and ingredients. The session covered topics like gene editing and modification, plant-based proteins, and alternative protein sources. Once again, the student worked across sectors and disciplinary boundaries, with lawyers, geneticists, academics, advocates, food scientists, agronomists, and nutritionists to develop key issues and stakeholder priorities. These priorities were subsequently presented to the FDA.

Table 7.2 – Domain 2: Non-government / non-profit

Domain 2: Non-government / Non-profit					
Location of experience	Role	Sector engaged	Key Activity	Disciplines	Learning outcome
Whole grain non-profit organization	Intern	Non-government/non-profit, industry, government	Whole grain regulations and supply chain assessments	Food science, nutrition, regulatory, processing, public health	Understanding how regulations influence food industry processes, understand how the supply chain influences consumption
Institute on Science for Global Policy	Adjunct fellow	Non-government/non-profit, industry, government	Reviewed and edited position papers on biotechnology in the food industry, facilitated caucus discussion	Food science, nutrition, regulatory, processing, plant genetics, biotechnology	Understanding the relationship between science and policy, and external groups' influence on policy

Experiences in Domain 2 fostered T-shaped skills. The student applied disciplinary knowledge (e.g. food and nutrition science) in a practical context (e.g. grain supply

chains and ingredient innovation) while collaborating across disciplines and across sectors. Critical thinking, communication, and collaboration were essential for bridging disciplinary gaps and discussing emerging food systems issues.

7.3.2.1 Linkages to other domains and activities

Experiences in the non-government/non-profit domain allowed the author to further develop an understanding of the influence of policy and regulations on food systems. Perhaps more importantly, the author observed first-hand how diverse stakeholders subtly influence policy processes. For example, stakeholders with a vested interest in plant-protein companies may advocate for policies that support increased consumption of these protein sources. This domain also provided perspective regarding the influence of the supply chain on consumption. For example, under-developed supply chain processes may decrease availability of healthful products, which reduces consumption.

7.3.2.1 Key lesson

Projects within this domain occurred at the nexus of the industry, government, NGO, and academic sectors. The cross sector, cross disciplinary collaborations in this domain provided insight into the complexity of food systems issues. Specifically, these experiences further strengthened the author's ability to understand how changes in one part of the food system produce downstream implications for other parts of the food system.

7.3.3 Domain 3: Research and development

Domain 3 encompasses the research activities, either occurring in industry, academia, or as a collaboration between the two, that contribute to the development of foods or crops.

The primary experience of Domain 3 is summarized in 7.3. The student engaged in a wide range of research and development activities throughout graduate school in the Department of Food Science and Nutrition at the University of Minnesota. The graduate research project focused primarily on developing a new, sustainable crop for food use. The experience exposed the student to a wide range of research approaches spanning the supply chain.

By design, research and development experiences were nested throughout the supply chain to provide a more holistic, systemic perspective of new crop development. This process involved soft-systems modeling (SSM) exercises, which provided a broad understanding of the new-crop development process. SSM approaches have been used in other disciplines and sectors, such as the medical sector, to understand complex, ambiguous challenges (Augustsson et al., 2019). Briefly, the SSM approach outlines key relationships, interactions, processes, challenges, barriers, and opportunities throughout systems. Figure 7.3 illustrates a concept map as an example output from an SSM exercise applied to pennycress development. This process provided a holistic understanding of the larger system and helped to prioritize key technical components. One of the key technical components included improving the chemical composition of the seed to more closely align with food-grade standards. Here, the author worked with a multi-disciplinary team, including plant geneticists, plant breeders, and biochemists, to identify genes involved in biochemical pathways underlying the accumulation of toxic compounds (see chapter 4 for reference). Another critical component included performing an economic analysis of crop production. Here, using canola as an analogue for pennycress, the author

collaborated with applied economists to develop an enterprise budget estimating per-acre expenses and returns of production. The team also modeled potential impacts of a three-crop rotation. (see chapter 5 for reference). The project also fostered an understanding of the regulatory processes required to prime a new food ingredient for the market, such as the Generally Recognized As Safe (GRAS) process. While volume of food grade pennycress is limited, pre-emptive GRAS strategies were developed by multi-disciplinary teams including geneticists, breeders, agronomists, food scientists, nutritionists, and toxicologists throughout academia and industry. Ultimately, the SSM approach provided context for the technical components of the project and their interactions with other parts of the system.

Experiences in this domain fostered T-shaped skills and provided a holistic view of food systems processes required to develop new food crops. Furthermore, the student developed scientific and technical skills through hands-on, multi-disciplinary experiential learning. The flexible and cross-disciplinary nature of the project provided unique lessons. For instance, by collaborating with plant geneticists and plant breeders, the student learned the influence of upstream supply chain actions (e.g. genetics) influence downstream supply chain outcomes (e.g. ingredient quality). Collaborating with applied economists allowed the author to critically assess production economics and understand how economic outcomes influence producer decision-making. Engaging with cross-disciplinary, cross-sector stakeholders provided more insight into the influence of regulations on the value chain, which built upon, lessons learned in Domains 1 and 2.

Notably, each of these experiences occurred in a multi-disciplinary, multi-sector setting. Members of both academia and industry intimately collaborated on each element of this project, which provided a greater understanding and appreciation of linkages and relationship between the two sectors. Perhaps most importantly, the project allowed the author to understand different mindsets and approaches of different disciplines and sectors. This is a crucial lesson as different disciplines and sectors approach problems from different perspectives. Fostering mutual understanding to cultivate shared value and common purpose between potentially disparate groups is a crucial skill for managing and accommodating 21st wicked food systems problems.

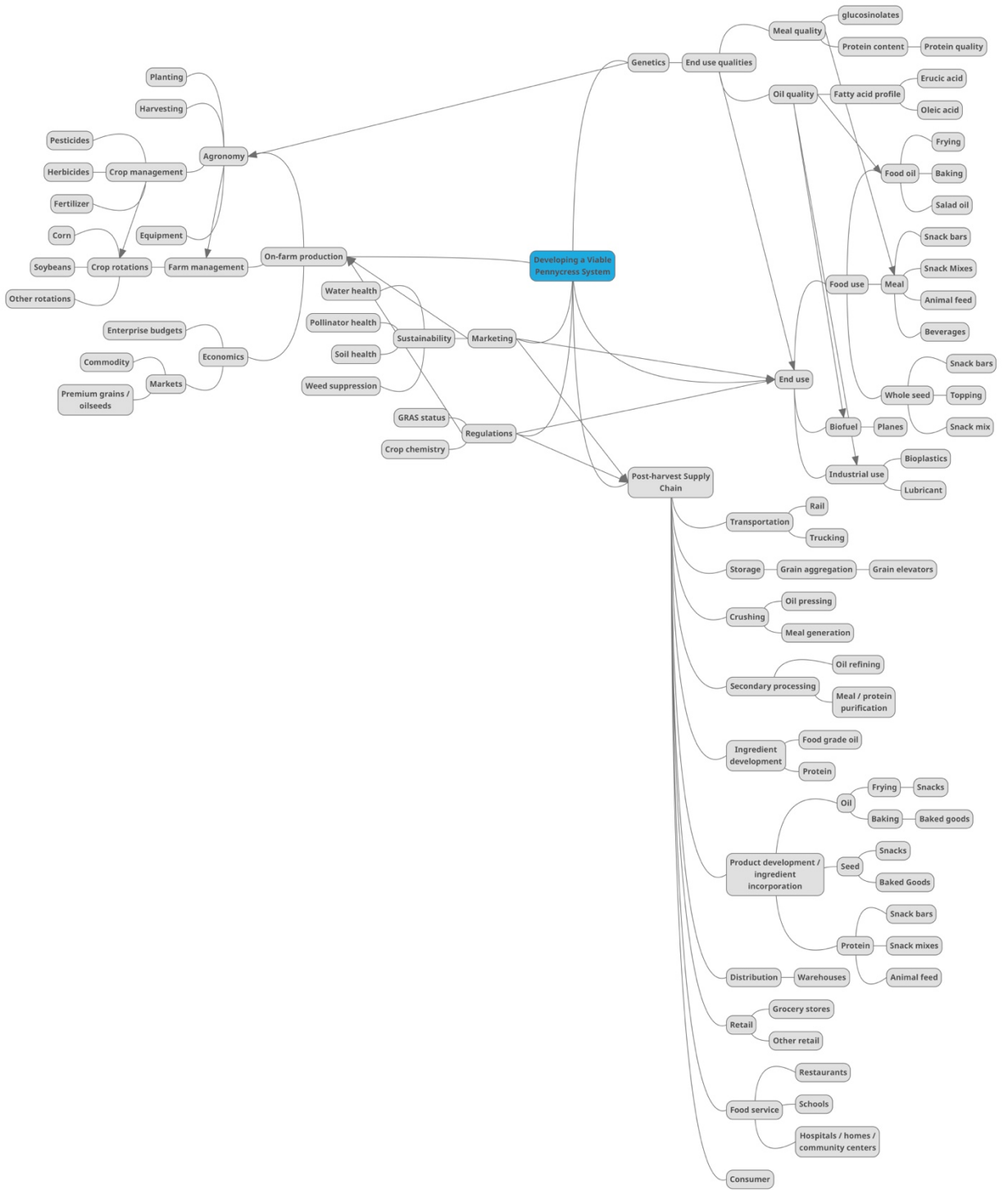


Figure 7.3 - A systems map for pennycress development

Table 7.3 – Domain 3: Research and Development

Domain 3: Research and Development					
Location of experience	Role	Sector engaged	Key Activity	Disciplines	Learning outcome
University of Minnesota	Graduate Researcher	Industry, academia	Researched crop improvement: genetics and composition, production economics supply chain and systems mapping, regulations	Food science, nutrition, regulatory, plant genetics, agronomy, processing, applied economics, public health	Understanding how upstream value chain processes (e.g. genetics) influence downstream applications, regulatory influence on value chain, role of production economics on decision making processes

7.3.3.1 Linkages to other domains and activities

Domain 3 provided a unique experience at the intersection of environmental and food issues. While a traditional nutrition graduate program might focus solely on the food or health potential of a product or ingredient, this program nested technical aspects (e.g. genetic/compositional improvement, economic analyses) within the context of the food system. Developing a food-grade pennycress has the potential to satisfy the environmental, economic, societal, and health components of sustainability by minimizing environmental impacts, providing a food source, contributing to the economic prosperity of producers, and promoting societal wellness. This type of food solution is essential to manage 21st century wicked food systems problems, like the diet-health-environment trilemma.

7.3.3.2 Key lessons

The integrated, multi-disciplinary approach to research and development allowed the author to place technical knowledge into a practical context. For example, understanding

the genetic processes underlying the accumulation of toxic compounds is essential to develop food grade pennycress. Developing food grade pennycress unlocks an end-use, thus providing a market for the crop. The supply chain must be developed to deliver the crop from the farm to processors and ultimately to consumers within the food environment. The raw material must comply with regulations to enter the market. Once these system requirements are satisfied, more producers might choose to grow the crop, which has the potential to improve the environmental impact of agricultural practices. Ultimately, the experience strengthened the ability to conceptualize complex food systems problems, identify critical points of intervention, and develop strategies to enact systems-based solutions.

7.3.4 Domain 4: Civic engagement / community

Domain 4 encompasses the activities that advance public or community concerns. In this context, community refers to a group of people living in the same geographic location or sharing a common set of characteristics. The primary experiences of Domain 4 are summarized in Table 7.4. Communities play an important role in food systems. For instance, socio-cultural norms, physical infrastructure, and economic status influence critical food systems outcomes like availability, access, consumption, and ultimately, public health (Aranceta, 2003). Therefore, pursuing activities in this domain was crucial to provide a holistic understanding of the food system. Here, the author engaged in two specific activities aimed at better understanding diverse socio-cultural food systems perspectives and incorporating community voices into the local food system.

The student first engaged in community learning by founding and serving as the president for a student group called the Student Ambassadors of Food, Health, and Culture. The group emphasized cultural diversity and provided an opportunity for students to expand their understanding of the relationship between food, health, and culture as these topics are largely absent from the standard nutrition and food science curricula. While the group was housed in the Department of Food Science and Nutrition at the University of Minnesota, students from multiple disciplines, including animal science, plant science, agronomy, public health, and business also participated. Beyond providing a space for students to explore cultural diversity within the food system, the student group also influenced several departmental initiatives by providing a platform for students to engage with faculty. The group also facilitated university-wide programs, like the Partnerships for a Sustainable Food System event. This event convened diverse stakeholders, from community organizations, such as food banks and food hubs; food service professionals, including chefs; and local farming groups, to discuss the role of University-community partnerships in creating a more equitable, sustainable food system. This event, along with other group activities, provided a mechanism to reach out to diverse stakeholders and constituents throughout local communities.

The student collaborated with the Minnesota Student Association to form the Dining Subcommittee, which provided food service recommendations to University administrators based on student dietary requirements. During this period, the University sought to amend their campus food service contract. The Minnesota Student Association aimed to incorporate student voices into this process and provided recommendations based on

student concerns. The student led a working group focused on dietary restrictions. This process exposed the author to the role that culture plays in dietary patterns and food choice. Beyond dietary restrictions, students felt strongly about the nutritional content of their food, as well as the ethical and sustainability implications of local and conventional food systems. These suggestions were compiled into a report that was presented to university administration.

Experiences in this domain fostered T-shaped skills and provided a holistic view of the relationship between communities and the food system. Here, cross-cultural engagement, empathy, and integrity were essential for creating a shared understanding between the student, community members, and other food systems stakeholders. These skills are essential to ensure that food systems outcomes are culturally appropriate and equitable.

Table 7.4 – Domain 4: Civic Engagement / Community

Domain 4: Civic Engagement / Community					
Location of experience	Role	Sector engaged	Key Activity	Disciplines	Learning outcome
Student Ambassadors of Food, Health, and Culture	Founder, president	Academia	Developed programming and hosted guest speakers to discuss the role and influence of community and cultures on the food system	Food science, nutrition, animal science, plant science, public health	Understanding the influence of culture and community on the food system; experiencing diverse perspectives and mindsets related to food
Minnesota Student Association	Dietary recommendations working group lead	Academia	Developed dietary recommendations for university administration based on a diverse constituency of students	Food science, nutrition, animal science, plant science, public health, environmental studies, applied economics, public policy	Understanding the influence of a diverse constituency and their diverse dietary needs on institutional policies and food offerings

7.3.4.1 Linkages to other domains and activities

Communities are intimately integrated with other sectors and processes described in the previous domains. For example, consumer advocacy NGOs, which represent community voices, influence policy decisions that result in regulations. These regulations influence supply chain and research and development activities. Supply chains ultimately determine the accessibility, availability and overall quality of food served in eating environments, such as restaurants, retail, or other food service operations, located in communities.

7.3.4.2 Key lesson

These experiences provided platforms to integrate community views into institutional processes, which served as a mechanism to identify critical issues within the student / university community. Ultimately, this approach helps better serve constituents. These processes provided a holistic understanding of the influence of culture and community on key food system drivers, like consumption, dietary quality, and institutional processes. Consumer needs, desires, and acceptance largely drive the food system, including institutional, policy and supply chain decision-making processes. Therefore, community outcomes are closely integrated into food systems activities.

7.4 Discussion: Key abilities for food systems leaders

This training program exposed the student to a wide range of experiential learning opportunities through inter-sector collaborations and direct engagement with multiple disciplines. The program provided the student with a broad view of the roles, functions, and activities of a diverse array of key food system players. This dynamic real-world

structure was designed to account for the growing interconnectedness of the food system (Nyström et al., 2019). This structure was instrumental to facilitate cross functional collaborations with diverse disciplines, like plant genetics, agronomy, economics, regulatory, and business as these collaborations are becoming crucial to thrive in the global food system (Tu et al., 2019).

Systems approaches to research and training that integrate diverse sectors, disciplines, and cultures throughout the food system are essential to manage wicked food problems, like malnutrition and environmental degradation. Conceiving and implementing systems approaches that integrate food systems components and drivers will require capacity building within the fields of agricultural, food, and nutrition sciences.

While experts across food systems disciplines have identified a need to implement systems-based education, there are few practical examples that engage across fields, from agricultural sciences (e.g. plant science, agronomy) to downstream fields (e.g. economics, social, food, and nutrition science). This case provides a practical example of a training program that specifically integrated diverse food systems components with the intent to work across disciplines and sectors to address 21st century, wicked food systems problems.

This multi-disciplinary, multi-sector training approach strengthened key abilities beyond the core technical skills instilled through a graduate curriculum. These abilities were

sequentially refined and built-upon throughout the learning experiences. Based on this learning, we discuss three key abilities essential for food systems professionals.

7.4.1 Key ability 1: conceptualize food systems

The food system is a complex, adaptive system. Actions at any one part of the system cascade and produce downstream consequences. Therefore, it is essential for agriculture, food, and nutrition professionals to conceptualize the complex linkages and relationships throughout the food system. Understanding these relationships and the downstream implications of component interactions requires critical thinking and systems thinking, two attributes on the horizontal axis of the T-shaped professional in Figure 7.1. There are many models and interpretations of the food system that differ depending on stakeholder mindsets, world views, assumptions, and boundaries. Based on the experiential learning activities pursued in this program, the student developed the food system conceptual model illustrated in Figure 7.4. This model provides a broad overview of a generic food system.

In this model, the supply chain sets at the core of the food system. The supply chain includes all the activities and processes between plant breeding, agricultural production, food manufacturing, retail, food service, and consumption. This chain is influenced by several key drivers. Biophysical environments determine where and how supply chains operate. In turn, the supply chain often uses natural resources which influences ecosystems. Natural resource availability along with pressures within the ecosystem can then influence supply chain efficiency and effectiveness.

Science and technology enable innovations, which can influence supply chain efficiency and capability. For example, innovations in plant breeding have produced foods with improved nutrient content and processing qualities. Governments and regulatory bodies enact policies and regulations, which set boundaries and limitations for supply chain operations. These can include food safety regulations, which protect the integrity of the food supply. These policies are often influenced by external groups, like NGOs or industry lobbyists. Social norms and societal factors also drive supply chain outcomes as these factors are crucial for consumer acceptance. For example, recent societal trends have driven the localization of supply chains and the increased production of organic foods. Industries, markets, and economics play critical roles in supply chain outcomes. For example, supply chains success is predicated on the delivery of economically viable products into established consumer markets. As illustrated in Figure 7.4, each of these factors are interlinked, and relationships are bidirectional.

The confluence of food system interactions influences food and environmental security. The food system determines availability, accessibility, affordability, nutrient content, quality, practicality, and safety of food sources. Food systems practices also influence environmental factors, like soil health, water quality, biodiversity, and air quality. In turn, food security and environmental sustainability often serve as feedback mechanisms, which influence markets, policies, technological innovations, and societal trends. Solving 21st century wicked problem, like the triple burden of malnutrition and environmental degradation requires food systems professionals to understand these interlinkages, relationships, and feedback mechanisms.

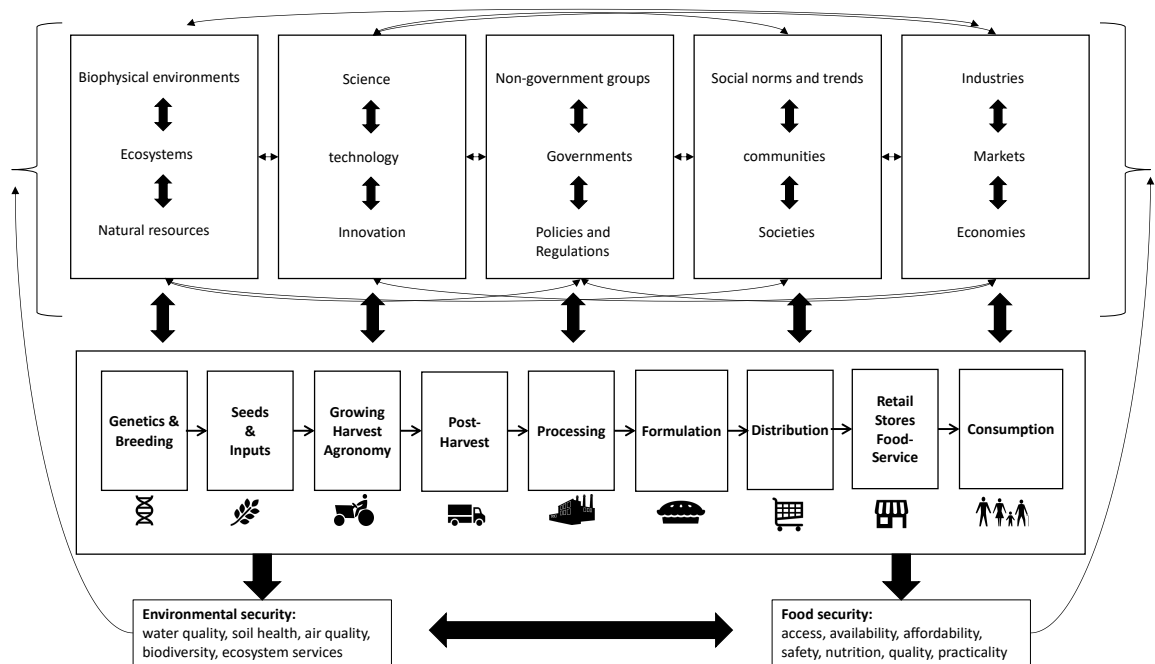


Figure 7.4 - Food system concept map

7.4.2 Key ability 2: identify critical development points (CDPs) in food systems

Conceptualizing food systems can help identify and prioritize critical development points (CDPs) throughout the system. Domain 3, research and development, provides an example of identifying CDPs and executing an action plan to address the problem. Figure 7.3 presents a mind map of the new crop development process to illustrate a practical example of an interlinked food systems problem, related to the development of pennycress. Glucosinolates limit end-use quality of brassica meals, and regulatory standards dictate that food-grade oilseeds contain $<30 \mu\text{mol/g}$ of glucosinolates. This demonstrates how end-use qualities and health outcomes influence regulatory processes.

Reducing glucosinolates to comply with regulatory standards requires an understanding of the genetic processes underlying glucosinolate accumulation. Improving the composition of pennycress products (e.g. oil and seed meal) can unlock end use market opportunities. Developing a market that supports the production of pennycress may have the downstream benefit of improving environmental sustainability. Beyond compositional improvements, more work is needed to assess pennycress production economics. Understanding on-farm production economics can provide orientation for other parts of the supply chain. Economic viability is essential for the production, delivery, and consumption of agricultural products and foods. Addressing these topics requires a multi-disciplinary approach.

7.4.3 Key ability 3: collaborate across disciplines to address systems problems and issues

Managing systems problems requires cross-disciplinary, often cross-sector, approaches. As illustrated in figure 7.4, food systems relationships are bidirectional and complexly interlinked. Therefore, actions in one area of the food system produce downstream consequences. Cross-disciplinary collaborations are essential to understand and manage downstream implications of technical, regulatory, and economic actions throughout the overall system. Therefore, food systems professionals must be trained in cross-disciplinary concepts, and they must be trained with the character attributes and skills necessary to facilitate a successful partnership.

Collaborating across disciplines requires the individual to learn, often experientially, to engage unfamiliar disciplines. In our case, the author, a nutrition scientist by training,

engaged and actively collaborated across disciplines throughout each domain, as illustrated in tables 1-4. Specifically, the author collaborated with plant geneticists and economists to solve two technical problems related to pennycress development. Facilitating successful cross disciplinary collaborations requires several skills that align with the horizontal axis of the T-shaped model. For instance, critical thinking along with systems thinking were essential in analyzing and engaging stakeholders in each of the four domains. These skills helped the author establish foundational knowledge prior to engaging stakeholders to develop problem-solving strategies. Open-mindedness and flexibility were essential for learning new techniques and approaches across disciplines. During these collaborations, communication and accountability were critical factors for ensuring trust within and across the teams. Understanding and appreciating these T-shaped attributes was essential for ensuring the ongoing success of the cross-disciplinary collaborations to manage food systems problems throughout the case.

7.5 Essential elements for food systems learning

Creating and implementing food systems-based training programs are challenging due to the large scale, complexity, and often ambiguous nature of food systems. Based on our experiences, our academic team identified key attributes in the student, advisor, academic team, learning environment, and projects that were helpful in facilitating our program. In fact, one might consider the confluence of these elements the “perfect storm” for food systems learning, as our program involved the right student, the right academic advisor, the right academic team, working on the right projects, in the right environment, at the right time.

First, food systems learning projects are dynamic, flexible, iterative, and experiential in nature. Learning activities occur in ambiguous, real-world environments, which are often un-defined. Given the wickedness of the food system, these projects often lack clear direction and standard solutions, which incentivizes the student, advisor, and other collaborators to intimately work together to accommodate diverse mindsets and approaches to manage challenges. Therefore, the advisor, student, and academic team must trust, share common values, and possess a level of comfort with the unknown while navigating ambiguity, uncertainty, and risk associated with these projects. Failures often occur in real-time, so the academic team must possess flexibility and mental agility to quickly manage emerging issues.

The learning environment is critical in facilitating systems-based projects. In our case, the University of Minnesota provided a rich environment for cross-disciplinary training. The University of Minnesota College of Food, Agriculture, and Natural Resource Sciences and the College of Biological Sciences together represent over 20 food systems disciplines, spanning plant sciences, agronomy, food science, nutrition, biosystems engineering, microbiology, molecular biology, environmental sciences, animal sciences, public health, and applied economics. This dynamic environment allowed the student to experience a more holistic view of the food system while actively participating in the development and application of novel approaches and techniques. Since the University of Minnesota is situated in a regional food hub, the student was able to apply these lessons in a practical context in cooperation with food systems actors. Minnesota hosts several major food and agriculture companies, such as General Mills and Cargill, as well as many

smaller, entrepreneurial companies. Interactions with these firms exposed the student to practical food systems situations and contexts that are not often accessible to graduate students. Taken together, this environment allowed the student to pursue cross-disciplinary training alongside world class scientists, while concomitantly applying that training to practical situations in companies and non-profits within the Minnesota food system.

7.6 Conclusion

Many professionals recognize the need to cultivate systems thinking and training to develop the next generation of food systems leaders, yet few models exist that emphasize this approach. Here we presented an example of a potential systems-based training model to integrate food systems disciplines. Our model integrated several disciplines, sectors, and learning experiences that exposed the author to a diverse array of roles, functions, and activities throughout the food system.

The experiences described in this chapter provide a road map for future students to pursue systems-based learning in food, agriculture, and nutrition sciences. We emphasized the importance of four different domains for food systems learning. While these domains are not comprehensive, they provide a wide range of experiential learning opportunities, which exposed the student to many different sectors, disciplines, roles, functions, and activities throughout the food system. Hands-on learning within each domain allowed the author to actively participate in major food systems-issues, often seeing progress in real time. Future food systems learning programs may find it beneficial to alter these domains to better suit the natural inclination, specific situations, wisdom of

the participants, and location of the program. In this case, integrated food systems-learning and research provided a platform for the student to actively participate in food systems change. Taken together, these experiences fostered capacity across research and development, regulatory, NGO, and community domains. Creating professionals with systems-wide experiences in these domains may foster future benefits for the economy, environment, health, and society. Understanding these diverse components can aid in the development of comprehensive, holistic, systems-based approaches to effectively manage 21st century wicked food systems problems.

7.7 Relevance to the nutrition ecology framework and the food system

The interdisciplinary training model was designed to expose the student to diverse stakeholders and food system components. The experiential learning activities included elements of each component of the nutrition ecology framework. The activities in the policy/regulatory and NGO domains aligned with the health, society, and economic components of the nutrition ecology framework. Additionally, activities in the research and development domain aligned with the health, economic, environmental, and societal domains of the nutrition ecology framework. Likewise, activities in the civic engagement/community domain aligned with the health and society component of the nutrition ecology framework. All activities ranging from regulatory compliance, new-crop development, supply chain analysis, and community engagement contributed toward the development of more sustainable, healthful, equitable food systems.

Chapter 8: Conclusions – Toward the formalization of systems-based agriculture, food, and nutrition approaches

8.1 Introduction

The food system sits at the intersection of agricultural production and human health. This system includes all the actors and processes involved in producing, delivering, and consuming foods, including the food supply chain, food environments, and consumer behaviors (Drewnowski et al., 2020). Supply chain processes, like plant breeding, agricultural production, research and development, food manufacturing, transportation logistics, and storage influence availability, affordability, and access to foods (C. Hawkes & Ruel, 2012). The food environment, sometimes referred to as the eating environment, is the human-built physical and social environments that impact accessibility, availability, and adequacy of food within a community or region (Rideout et al., 2015). Common food environments include restaurants, homes, and food service operations, like hospital or school cafeterias. Consumers behaviors, such as purchasing, preparing, and consuming foods, are largely based on food availability determined by supply chains, and eating environments. Food systems actors are influenced by external drivers, like policies and regulations, technological advances, economic and market factors, biological and physical environments, and societal trends (HLPE, 2017). Policies often determine funding for food system activities and set priorities for agricultural production. For example, in the United States, the Farm Bill determines producer subsidies and provides funding for critical nutrition programs, such as the National School Lunch Program (Echon, 2014). Regulations, such as food labeling, food safety requirements, standards of identity, influence food systems processes (Campos et al., 2011). For example, food safety regulations ensure that supply chains produce safe foods and labeling regulations to help consumers make informed decisions. Technological innovations can improve the

efficiency of production and delivery of foods. Economic and market factors influence food production, availability, and affordability (Lusk et al., 2011). Biological and physical environments influence how and where food is produced. For example, climate plays a major role in determining where crops are grown throughout a region.

Additionally, access and readily available resources (e.g. water) determine where foods are produced. Finally, societal trends drive production and consumption. For instance, the increased interest in plant-based and non-animal protein has driven the increased production of meat alternatives. Clearly, food systems actors and drivers play critical roles in the production, delivery, and consumption of foods.

The food system is complex and adaptive. Complex, adaptive systems are non-linear, involve many different components, and produce many different outcomes (J. H. Miller & Page, 2007). Changes to one part of the system have rippling effects throughout other parts of the system. While individual actors (e.g. supply chains) greatly influence food systems outcomes, the interaction between actors and drivers (e.g. policy, economics) produces large, complicated food systems outcomes (Corinna Hawkes, 2009). For example, the increased interests in non-animal protein has created new technologies, new products, new markets, new supply chains, and new businesses. Working together, these components have increased interest in and consumption of non-animal proteins. These components have also influenced regulations throughout the food sector (see recent discussions around defining meat analogs, and non-dairy milks, for example). The complex, interlinked nature of the food system produces adaptive, wicked challenges.

Wicked challenges are complex and interlinked; involve many stakeholders, sectors, and disciplines with potentially differing worldviews; and lack clear cut, definitive answers (Rittel & Webber, 1973). Wicked problems contrast with “tame” problems, which have definitive solutions. Tame problems are often described as problems that have one-to-one cause and effect relationships whereas many factors contribute to wicked problems. While tame problems may be solved by a single discipline or sector, wicked problems require multiple-disciplinary, multiple-sector systems approaches (Dentoni et al., 2012). The diverse disciplines, sectors, and stakeholders within the food system create a ripe environment for wicked problems. Granted, there are many tame problems within the food system, these often occur within a wicked context. For example, dietary deficiencies such as goiter and pellagra occurred at epidemic levels in the United States in the early 1900s (WHO, 2000). One might consider the root cause of dietary deficiencies as a tame problem: eventually, nutrition scientists identified the nutrients responsible for these diseases (e.g. iodine deficiency for goiter, niacin deficiency for pellagra). However, implementing a solution at scale required a complex, interlinked approach involving several disciplines, sectors, food systems actors, and drivers. Now, policies requiring enrichment influenced the food supply chain to produce iodized salt and enriched flour, making them widely available for consumption, effectively reducing goiter and pellagra (Clay, 2018). The tame problem, i.e. nutrient deficiency, occurred in a wicked environment, i.e. the food system. Solving this problem required a systems approach that engaged policy and supply chains to increase consumption of nutrients of interest. In the 21st century, food systems challenges are more wicked than ever before, and solving

these problems requires a workforce of nutrition professionals trained to develop and implement systems approaches.

The global food system contributes to two interlinked, strikingly wicked problems: malnutrition and environmental degradation. Currently, the global population faces the triple burden of malnutrition: hunger, micronutrient deficiencies, and overweight and obesity. Across the globe, more than 820 million people experienced chronic hunger (FAO, 2018), 2 billion experience micronutrient deficiencies (WHO, 2018), and 2 billion people experience overweight or obesity (FAO, 2018). This is largely due to a food system that, in some cases, fails to deliver sufficient energy and micronutrients, and in other cases, produces inexpensive, high-calorie, low nutrient-dense foods. Meanwhile, the same food system contributes to environmental degradation. The global food system is responsible for nearly 30% of all greenhouse gas emissions and accounts for 40% of all freshwater use (EPA, 2020). Poor food production practices also contribute to decreased biodiversity and water eutrophication. The relationship between the food system, malnutrition, and environmental degradation has been referred to as the “diet-health-environment trilemma,” and the negative implications of this system may exacerbate as the population exceeds 9 billion by 2050, and food production increases to satisfy demand (M. Clark et al., 2018). Multi-pronged, multi-disciplinary systems approaches that account for the health, ecological, economic, and social impacts of food solutions are required to address these challenges.

Many food, nutrition, and agriculture professionals recognize the need to implement systems approaches to address the complex, wicked food system challenges. For instance, Lee and colleagues suggest a systems framework, that targets policy, nutrition, healthcare, and economics, is essential to manage the obesity epidemic (Lee et al., 2017). This process involves integrating multiple disciplines and targeting multiple levels of complexity, ranging from individual genetics to biophysical environments and societal norms. Jordan and colleagues suggest the sustainable commercialization of new crops, using a systems approach, can preserve the integrity of the environment and the economy (N.R. Jordan et al., 2016). This approach integrates multiple disciplines and stakeholders to design new agroecosystems and to develop supply chains, end uses, and markets for crops within these systems. Other studies have implemented nutrition sensitive supply chain approaches targeting agricultural production, consumption, and rural economic development (S. Allen & Brauw, 2018; Summer Allen & Brauw, 2018; Brauw et al., 2015). These approaches have been successful at increasing production and consumption of nutrient-rich foods, such as orange flesh sweet potatoes and iron fortified milk, in developing countries (Finkelstein et al., 2015; Hotz et al., 2012). These approaches align with the nutrition ecology framework, which considers environmental, economic, health, and social factors co-equally throughout the food system (Raiten & Combs, 2019; Schneider & Hoffmann, 2011). While many systems-based frameworks exist, implementing systems approaches requires training and capacity building within the fields of agriculture, food, and nutrition.

Using systems approaches to solve wicked food systems problems requires new approaches to training agriculture, food, and nutrition professionals. Leaders within these fields recognize the need for multi-disciplinary, systems-based training. A report by the High Level Panel Experts on Food Security and Nutrition suggest that building human capacity across nutrition and food systems is essential to translate evidence into action (HLPE, 2017). They suggest that nutrition professionals must be trained in key concepts across multiple disciplines, like agriculture, policy, and environmental sciences (HLPE, 2017). Others suggest that balancing technical, managerial, and leadership capacity is essential to engage in multi-sector, multi-disciplinary partnerships required to scale-up nutrition solutions (Mucha, 2013). Fanzo and colleagues describe essential skills for nutrition professionals to thrive in a post-2015 environment, and these skills include communication, multi-sectoral collaboration, advocacy, leadership and technical skills (J. C. Fanzo et al., 2015). Hickson and colleagues suggest that adding more hands-on learning experiences and improve professional skill development within nutrition training (Hickson et al., 2018). Some reports provide frameworks to pursue multiple-disciplinary, systems-based training. Ingram and colleagues described the need for a workforce trained as food systems analysts to manage issues throughout the food system ranging from food security, environmental degradation, malnutrition, and economic disparities (Ingram et al., 2020). This report provides critical elements for food systems leaders including transdisciplinary collaboration, systems thinking, effective communication, and exposure to ways in which others conceptualize food systems (Ingram et al., 2020). Jordan and colleagues described a pilot program that exposes students to hands-on learning experiences in civil society, private enterprise, and government sectors with the goal of

creating and sustaining agro-eco-innovation (Nicholas R. Jordan et al., 2012). While experts across food systems disciplines have identified a need to implement systems-based education, there are few practical examples that engage across fields, from agricultural sciences (e.g. plant science, agronomy) to downstream fields (e.g. economics, social, food, and nutrition science).

There is a clear need for systems approaches in the fields of agriculture, food, and nutrition sciences. Cross-sector, cross-disciplinary approaches are essential to manage wicked food systems challenges. In order to effectively implement these approaches, our fields must evolve to train the next generation of professionals to work across disciplines and across sectors. This requires the development of technical, leadership, and managerial skills, similar to the T-shaped professional concept (Conley et al., 2017). The work presented in this dissertation aimed to address these gaps. First, it demonstrated a multiple disciplinary, cross-sector systems approach to understanding barriers, challenges, and opportunities throughout the whole grain food system. Then, it provided examples of cross-disciplinary research approaches to developing a new, sustainable crop for food use. Then, it discussed the role of the land grant university in developing sustainable, nutrition sensitive food systems. Finally, it provided a potential model for future food systems training based on experiential learning activities in four different food system domains. In conclusion, it highlights key lessons within a framework for future nutrition-systems approaches.

8.2 Outline for a nutrition systems framework

The projects and activities within this dissertation were designed to reimagine, catalyze, and transform current nutrition approaches and contribute toward establishing a systems-based framework for research, discovery, and renewed impact of the nutrition discipline. Systems-based approaches are often difficult to conceive and implement due to their complex, ambiguous nature. Therefore, we recommend a three-step framework based on our work, which can help catalyze systems-based approaches. This framework integrates systems mapping, stakeholder analysis and engagement, and technical & adaptive action to improve food systems outcomes. These steps create a deep understanding of the problematical situation, identify and prioritize critical development opportunities using a holistic lens, and ultimately execute a series of actions that can address technical and adaptive challenges. This framework is meant to be open and adaptive. Steps within the framework can accommodate social sciences and planning theory, food systems sciences, and business techniques to develop systems-based solutions. The following sections apply lessons learned from this dissertation to the recommended framework. These lessons provide practical examples of how these approaches might be applied to future agriculture, food, and nutrition situations.

8.2.1 Systems mapping

First, we recommend systems mapping as the first step in systems-based nutrition approaches. Systems mapping techniques create richer, deeper understandings of complex situations, which provides orientation to address problematic situations (P. B. Checkland & Haynes, 1994). For example, soft-systems modeling (SSM) is used in many disciplines, such as medicine and social planning research, to create a deeper, broader understanding of complex situations (Augustsson et al., 2019; P. B. Checkland &

Haynes, 1994). This method defines the problem, outlines intersecting systems and activities, and identifies possible, feasible changes to the situation. This step is potentially highly detailed and can include descriptions of transformational processes (e.g. systems actions, technical interventions, etc.), beneficiaries of transformational processes, actors involved in implementing change, owners, or those responsible for processes, the worldviews of engaged stakeholders, and potential biophysical, economic, or technical constraints of the situation (P. Checkland & Winter, 2006). This step can generate concept maps and other outputs that illustrate complex relationships and interactions throughout food systems. These tools help identify “pain points” and potential critical control points throughout systems, which can help target future transformational processes.

We produced systems maps for the whole grain and pennycress systems. Figure 7.3 provides an example of systems mapping output. Whole grains and pennycress are two potential food sources that can contribute to the health of the global population and environmental resilience. These two food sources are at vastly different stages of development. For decades, whole grains have been recommended as part of a healthy diet (Curtain & Grafenauer, 2020). However, despite years of research and development, whole grain consumption fails to meet recommendations established by the Dietary guidelines for Americans (Ahluwalia & Hughes, 2019; US Department of Agriculture and US Department of Health and Human Services, 2015). Systems mapping allowed our research team to identify system stakeholders to understand systemic barriers to whole grain availability in Twin Cities restaurants. These engagements are more thoroughly

described in the following section entitled ‘Stakeholder Engagement.’ In contrast to whole grains, pennycress is at an early stage of development. Pennycress has the potential to contribute to environmental sustainability by improving bio-dynamic, regenerative agriculture practices as a cash cover crop. The systems map shown in Figure 7.3 illustrates the intersection of activities and processes involved in pennycress development. Given its early stage in development, genetic improvements are essential for unlocking end-use and market opportunities that might influence producer adoption. Additionally, modeling on-farm production economics is essential to understand the economic impact of production and to prioritize future development opportunities (e.g. prioritizing genetic improvements that achieve breakeven yield, as described in chapter 5). In short, this step serves as a compass to identify milestones throughout the supply chain. Our teams pursued both of these activities, which are further discussed in the section entitled “Technical and adaptive action.”

8.2.2 Stakeholder analysis and engagement

We recommend stakeholder analysis and engagement as a second step in systems-based nutrition approaches. Using information generated during the systems mapping process, key stakeholders are identified throughout the food system. Stakeholders might include both food systems actors and drivers, such as plant breeders and geneticists, farmers, ingredient companies, food manufacturers, retailers, restaurants, policy makers, research and development scientists, policymakers, and consumer groups. Understanding the roles, functions, and activities of each stakeholder is essential to developing an engagement plan. Stakeholder engagement can occur through several processes. For example, research teams might engage stakeholders through interviews, focus groups, or

questionnaires. These qualitative methods dive deeper into critical issues facing food systems stakeholders. Other approaches, such as the Delphi method, might be used to gain anonymous perspective. The Delphi method is often used in the business sector to allow participants to freely voice perspectives around complicated issues (J. Skulmoski et al., 2007). Community-based participatory action research may also provide on-the-ground perspectives around critical issues faced by community stakeholders (Minkler, 2005). Taken together, these approaches create a holistic understanding of real-life issues and scenarios, which helps evaluate practicality and feasibility of potential transformational processes, as it relates to food and nutrition issues.

Practical example 1: whole grains

Key lesson: underdeveloped food system actors and drivers disincentivize whole grains consumption

Our research team identified key stakeholders throughout the whole grain food system and conducted interviews to create a richer understanding of critical barriers to whole grain consumption in restaurants. Stakeholders included grain millers, bakers, wheat geneticists, academic faculty, restaurateurs, chefs, and public health professionals. Based on stakeholder interviews, we conclude that cost is prohibitive to whole grain production and consumption, ambiguous regulations inhibit development of whole grain foods, supply chain challenges limit whole grain availability, and health stigma limits consumer desire to eat whole grains. Overcoming these barriers will require a coordinated, multi-sector, cross-disciplinary systems approach.

Opportunity for improvement and future perspectives

Increasing consumption of practical, healthful, affordable, desirable whole grain foods requires a multi-pronged, systems approach that targets food systems actors and drivers simultaneously to provide healthier, easier choices for consumers. Supply chain improvement activities should be targeted in a synergistic, synchronous manner. These activities might include breeding wheat to improve whole wheat flour quality and developing standard methods for whole grain processing, formulation, and testing. These processes might improve industry research and development capacity. Building supply chains that minimize costs for chefs and professionals in the eating environment could improve the economics of serving whole grain foods. Pursuing consensus amongst industry, academia, and government on a whole grain definition could eliminate ambiguities around formulating whole grain foods. Finally, allowing marketing campaigns to emphasize the benefits, rather than negative effects, of whole grain consumption could reduce consumer stigma around grain-based foods. Each activity described here represents a microcosm of a wicked food systems problem, as different sectors and disciplines have different mindsets, use different approaches, and have different incentive systems. However, overcoming these challenges and implementing this multi-disciplinary, cross-sector, systems approach could improve whole grain consumption, consumer dietary quality, and public health.

Practical example 2: pennycress

In contrast to whole grains, the stakeholder pool is smaller for pennycress given its early stage of development. Here, stakeholder engagement occurred through informal

processes rather than a research study. A multi-disciplinary team of plant geneticists, agronomists, food scientists, applied economists in academia and industry identified two technical challenges to unlock value chain potential for pennycress. These challenges included compositional improvement and on-farm production economic modeling.

8.2.3 Technical and adaptive action

We recommend technical and/or adaptive action as the third step in systems-based nutrition approaches. These actions enable transformational processes and addresses critical issues identified in the systems mapping and stakeholder engagement phases. This step can include a wide array of research approaches, from basic scientific research, behavioral interventions, modeling, or dissemination and implementation sciences (Brownson et al., 2017; Glasgow et al., 2012). Ultimately, these transformational processes can occur at any step in the system, with the ultimate goal of improving systems outcomes. While this is positioned as the final step in our recommended framework, we recognize that this process is iterative. Due to the adaptive nature of systems, outcomes from the action step may cause teams to reevaluate all processes (Figure 8.1). In the context of this research, our team pursued two technical projects with the goal of improving and better understanding the pennycress production system.

Practical example 1: pennycress genetics

Key lesson: understanding plant genetics can improve compositional quality toward unlocking end-use potential

Our stakeholder group identified two critical issues with pennycress: compositional improvement and economic modeling. Wild pennycress contains high levels of anti-

nutritional glucosinolates. These compounds reduce palatability of seed meal, the byproduct of the oil pressing process. High glucosinolate seed meal is associated with goiter and impaired thyroid function when fed to animals in high amounts. Thus, reducing seed glucosinolates is essential to valorize the pennycress system. Using a forward and reverse genetics approach, over 15,000 mutant pennycress lines were assessed for reduced glucosinolate levels. Low-glucosinolate mutants underwent whole genome sequencing to identify putative candidate genes responsible for glucosinolate accumulation. Lines carrying mutations in the transcription factor gene *MYC3* and the core structure biosynthesis gene *CYP83A1* exhibited ~45% and ~19% reductions in glucosinolates, respectively, compared to wild plants. Expression analyses revealed that other genes involved in glucosinolate biosynthesis and regulation were downregulated when these mutations were present. While more work is needed to understand exact genetic mechanisms underlying glucosinolate accumulation in pennycress, these genes are potential candidates to produce a viable food grade pennycress in combination with other beneficial genes.

Opportunity for improvement and future perspectives

Combining the *myc3* and *cyp83a1* low glucosinolate mutants with other beneficial traits can potentially lead to a viable, food grade pennycress variety. More work is needed to understand exact mechanisms underlying glucosinolate accumulation in pennycress. The work presented in this study is largely based on previous research in the model plant *Arabidopsis thaliana*, a close relative to pennycress. Similar results have been observed in *Arabidopsis*, yet there are potentially significant differences. For example, *MYC3* reduces glucosinolate content in *Arabidopsis* as part of the triple mutant *myc234*. These mutants

also experience disruptions in crucial development pathways, such as the jasmonic acid pathway, which can decrease plant fitness. In contrast, the single *myc3* pennycress mutant experiences a significant decrease in glucosinolates without decreasing plant fitness. This indicates that *MYC3* potentially plays an important role in glucosinolate accumulation. Additionally, altering glucosinolate biosynthesis and regulation may cause shifts in production of other metabolites. For example, amino acids that would normally participate in glucosinolate biosynthesis may be diverted to produce other compounds. This could influence end-uses for the crop. Additional work is also needed to understand how low glucosinolate mutants perform in on-farm production scenarios. Since glucosinolates defend against pests, reducing them may render the plants more susceptible to pests. This may result in an increase in pesticide application and input costs. Future genetic, agronomic, and economic studies can help improve our understanding on how these plants function in field production scenarios.

Practical example 2: pennycress economics

Key lesson: understanding canola production economics reveals potential for pennycress economic output

Our group modeled pennycress production economics based on canola data. As a new crop, more work is needed to understand potential economic implications of pennycress production. Our team assembled canola production data from farms in Minnesota and North Dakota during 2008-2017. Using the average canola price received by farmers as well as the average cost of production, we estimated net return of pennycress production based on a range of yields reported in the literature. In this model, our lowest yielding

estimate resulted in a net loss of \$152.90 per acre whereas our highest yielding estimate resulted in a net return of \$97.74 per acre. Based on the average canola price and expenses, pennycress must yield 1,495 pounds per acre for the operation to break even. We also estimated the potential economic impact of soybean yield reductions. Given pennycress has been reported to influence soybean yield, we estimated yield losses of 5% up to 30%. At a 5% yield loss, net return for soybean is reduced from the 10-year average of \$71.86 per acre to \$54.23 per acre. At a 30% yield loss, soybeans produce a net loss of \$34.59. Finally, we modeled the economic outcomes of a corn-pennycress-soy production system. Net return of the three-crop system ranged from a net loss of \$136.98 per acre to a net return of \$220.12 per acre. Based on these estimates, we conclude that pennycress yield is a critical factor in the profitability of pennycress as a single crop and as part of the three-crop system. Plant breeders should strive for a minimum yield of 1,495 pounds per acre for producers to break even. Potential soybean yield reductions are also critical to the profitability of the system. Plant breeders and agronomists should strive to minimize soybean yield reductions in the three-crop system.

Opportunity for improvement and future perspectives

Based on these estimates, we conclude that pennycress yield is a critical factor in the profitability of pennycress as a single crop and as part of the three-crop system. Plant breeders should strive for a minimum yield of 1,495 pounds per acre for producers to break even. Potential soybean yield reductions are also critical to the profitability of the system. Plant breeders and agronomists should strive to minimize soybean yield reductions in the three-crop system.

More work is needed to assess on-farm production economics of pennycress, as well as the potential impacts of the three-crop rotation. Our model used production data for a different crop, canola, and actual values for pennycress production will likely vary. For example, actual expenses of pennycress production may vary substantially from production expenses of canola. Additionally, canola price may not apply to pennycress. Given pennycress is not a traditionally recognized oil and protein source, it may fetch a lower price compared to canola. On the other hand, given its potential sustainability impacts, pennycress may fetch a premium price compared to other oilseeds. Finally, the protein content and quality as well as the oil profile may also influence price. For example, if pennycress has a higher, more bioavailable protein content it may demand a higher price in the market. Additionally, if pennycress produces a more desirable oil profile, it may demand a higher price than other oils.

Future work should assess the potential economic benefits of ecosystem services provided by pennycress. Given cover crops have the potential to reduce soil compaction, sequester soil nutrients, reduce water pollution, and suppress weeds, there may be economic benefits not captured by our model. More work is needed to understand how these ecosystem services contribute to the economic output of farm production. Having a better understanding of the economic impact of ecosystem services could influence producer adoption.

8.4 Future application of the recommended systems-based framework

The three components of the recommended framework are illustrated in Figure 8.1. A similar framework was applied to the development of a sustainable new crop, pennycress. Pennycress development activities occurred at the juncture between plant genetics, agriculture, economics, supply chains, and end-use. This work predominantly examined the impact of upstream supply chain activities (plant genetics) on downstream supply chain impacts (end use quality) and modeled production economics. These activities were chosen because they were key factors at the early stage of pennycress development. We identified gene targets with the potential to valorize the pennycress system by reducing anti-nutritive compounds and improving the food quality of pennycress seed meal. We also modeled pennycress production economics using on-farm production data for canola as an analog. Modeling on-farm production economics provides orientation for the first step of the supply chain and has the potential to prioritize future development opportunities. For example, future research might focus on developing pennycress lines that can achieve break-even yield. Future economic studies might elucidate the economic benefits ecosystem services provided by pennycress.

Future applications of this framework could occur at other points in the food system. Further work may also focus on convening and collaborating with a diverse array of food system stakeholders to pre-emptively identify potential challenges, barriers, and opportunities throughout the pennycress supply chain. For example, pennycress developers may convene current stakeholders throughout the oilseed supply chain, such as those listed in Table 8.1 to better understand potential pain points in the food oil supply chain. Once stakeholders identify potential opportunities, pennycress developers,

such as those at the University of Minnesota, can align around mutually beneficial research projects and broker collaborations to enable progress.

Figure 8.2 illustrates a hypothetical pennycress supply chain and identifies potential development opportunities. Moving forward, it may be critical to pre-emptively address these supply chain issues to better position pennycress for a smooth market birth. For instance, pennycress may contain other anti-nutritive compounds, which could limit market potential (Tyl et al., 2020). These may be quickly addressed based on existing genetic tools and resources (Chopra et al., 2018). Optimizing agronomics and understanding production economics of food grade varieties will also be essential for producer adoption. Optimizing post-harvest infrastructure, including shipping, storage, distribution, and processing capabilities will help further develop the supply chain. Understanding ingredient qualities and functional characteristics may help identify potential product opportunities. Developers should also target consumers to effectively communicate potential benefits of pennycress production and to understand potential acceptance of pennycress-based products. Perhaps these development opportunities should be approached from a cross-disciplinary, cross-sector systems approach similar to the approach described in this dissertation.

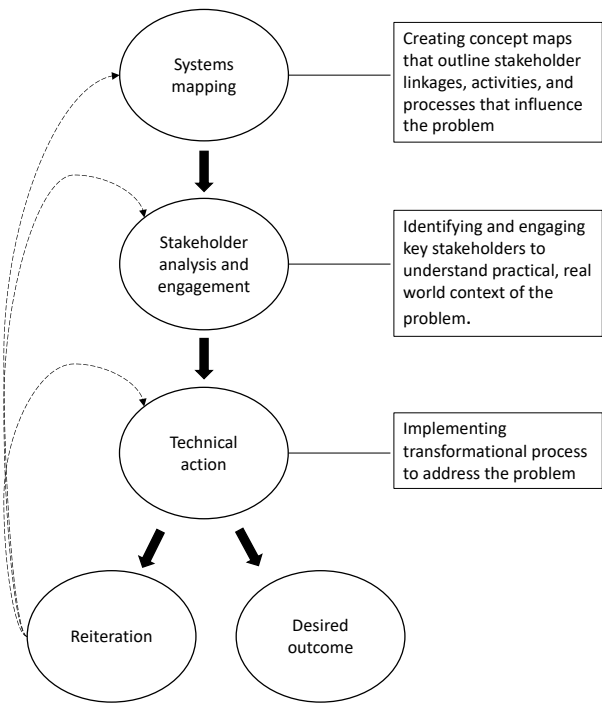


Figure 8.1 – three steps recommended when engaging in systems approaches

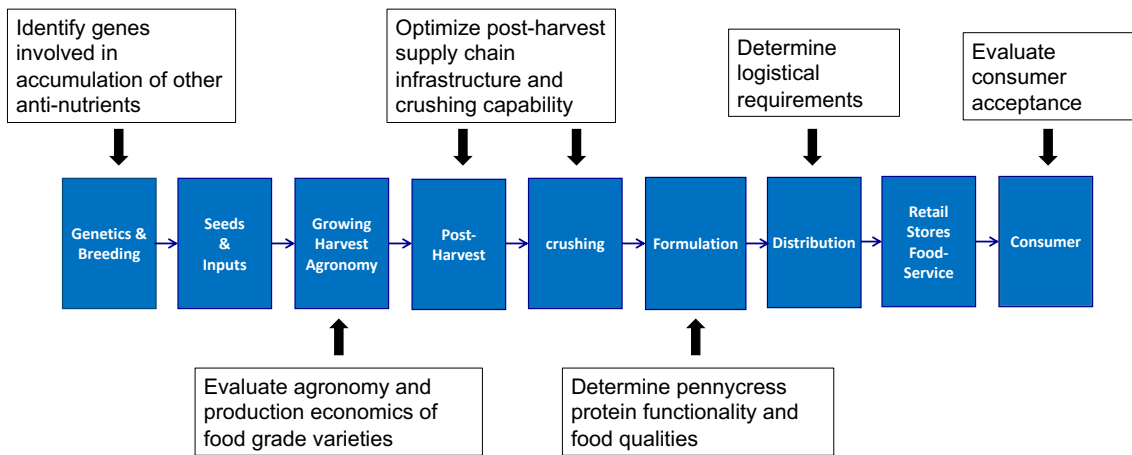


Figure 8.2 – potential development opportunities throughout the pennycress supply chain

Table 8.1: Canola supply chain industry stakeholders

Firm	Product	Location
ADM Processing	Crushing, seed, meal, oil	Velva, ND
Bunge Oils	Crushing, oil	St. Louis, MO
CHS, Inc.	Crushing, oil, meal	Kennedy, MN
Hart AgStrong LLC	Crushing	Danielsville, GA
Resaca Sun Feeds, LLC	Crushing	Resaca, GA
Viterra Oilseed Processing	Crushing	Warden, WA
Bayer CropScience	Seed	Research Triangle Park, NC; St. Louis, MO
Cargill	Seed, oil, meal	Minneapolis, MN
CROPLAN Seeds	Seed	Shoreview, MN
DuPont Pioneer	Seed	Mankato, MN
Mycogen Seeds (Corteva)	Seed	Indianapolis, IN
Rubisco Seeds, LLC	Seed	Philpot, KY

8.5 Relevance to the nutrition ecology framework

Nutrition professionals must work throughout the food system to develop sustainable, healthful food sources. Sustainable food sources must remain healthful, economically viable, environmentally resilient, and societally acceptable (Drewnowski et al., 2020). These elements align with the nutrition ecology framework (Schneider and Hoffman, 2011; Raiten and Combs, 2019). This work aligned with the nutrition ecology framework, most prominently exemplified by the pennycress development projects.

Pennycress provides an example of a potential sustainable food source. Figure 8.3 illustrates how pennycress aligns with all four elements of the nutrition ecology framework. We demonstrated cross-disciplinary, cross-supply chain improvement strategies by targeting plant genetics to improve the quality of raw materials and by modeling production economics. These approaches align with the nutrition ecology

framework by considering biological, environmental, economic factors in developing a sustainable new crop for food use. Figure 8.4 provides a graphical summary of this cross-supply chain approach.

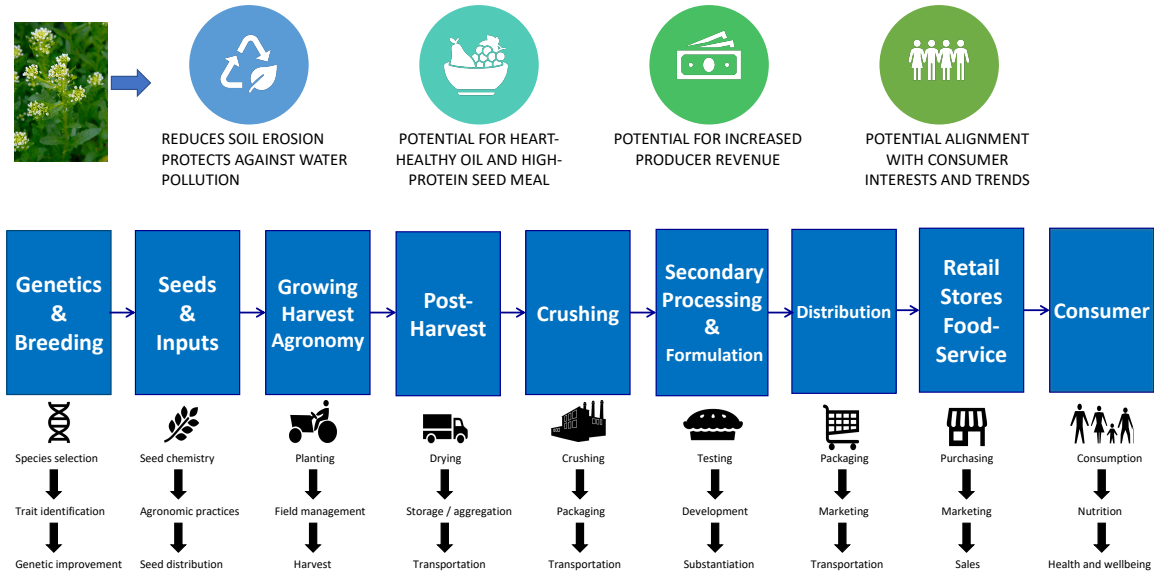


Figure 8.3 – pennycress aligns with the nutrition ecology framework

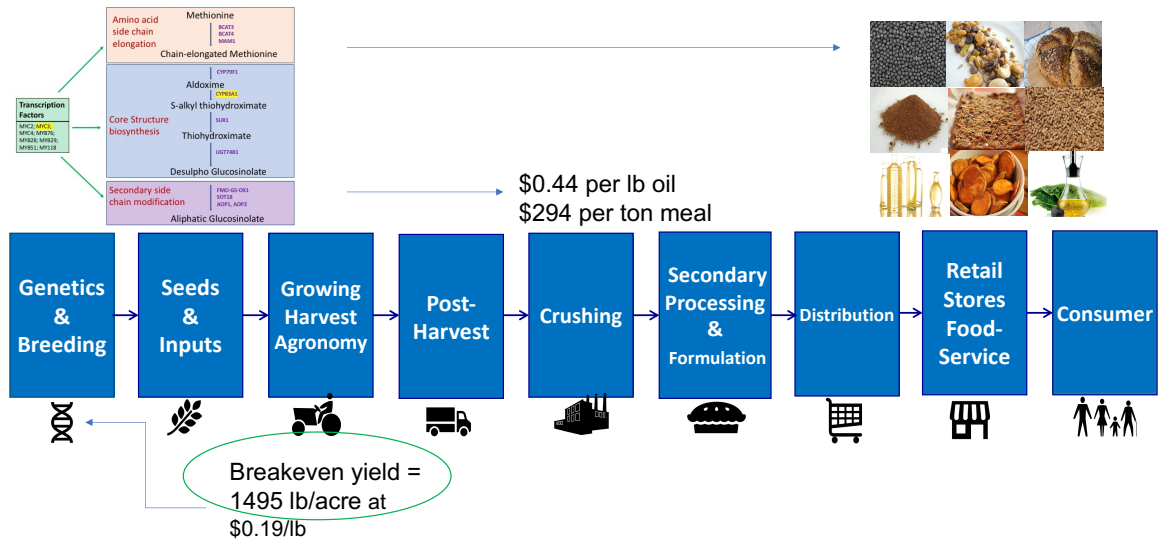


Figure 8.4 – cross supply chain approach to pennycress development. Improving composition using genetics unlocks downstream value and opportunities for end uses. Modeling economics helps determine breakeven yield, which provides a target for further improvement.

8.6 Interdisciplinary food systems training adds value to academia, industry, and society

The interdisciplinary food systems graduate research and training presented in this dissertation added value to academia, industry, and society. This added value is difficult to describe in the context of a typical research study, yet its impact has reverberated throughout several ongoing relationships and projects. Figure 8.5 illustrates this added value.

Our research and training provided a platform for industry-academia collaborations. The pennycress development project occurred as a collaboration between industry scientists and university faculty. Together, both groups provided insight regarding critical genetic targets and development processes and placed sustainable crop development into a

practical business context. Together, the stakeholders advanced next-generation agriculture practices and products; implemented innovative research in a practical context; delivered on public good aspects of a business mission, delivered on the land grant university mission, and implemented an innovative training model for the next generation of agriculture professionals.

Our projects also provided a platform for cross-college and cross-departmental collaborations. The plant genetics portion of this project was facilitated by collaborations between the College of Biological Science and the College of Food, Agriculture, and Natural Resource Sciences within the University of Minnesota. This included a strong working relationship and collaboration between the Department of Food Science and Nutrition, The Department of Agronomy and Plant Genetics, and the Department of Plant and Microbial biology. The economic modeling portion of this project occurred through a collaboration between the Department of Food Science and Nutrition and the Department of Applied Economics. Altogether, these projects contributed to the development of a sustainable crop for food use. This work also helped expand departmental capabilities. Prior to these projects, the Department of Food Science and Nutrition largely lacked a platform to engage with systems- based projects. As a result of this work, the department now has multiple platforms to engage in systems projects, including classes, agricultural systems research, and community-engagement programs. Each of these platforms helps advance the university mission of training, research, and outreach. Finally, this integrated approach increased exposure, relevance, and impact of the university. These projects have all been presented to international audience, and have helped university faculty,

staff, and students engage with international non-profits, government agencies, local non-profits, and professional societies.

Altogether, this work demonstrated an interdisciplinary approach to graduate research and training. Projects spanned the supply chain and engaged across sectors to develop a sustainable new crop. This served to create a framework to address systems-based problems. The research and training approaches fostered in this dissertation may provide a model for the University of Minnesota to pursue systems-based research and training toward the development of sustainable food systems.

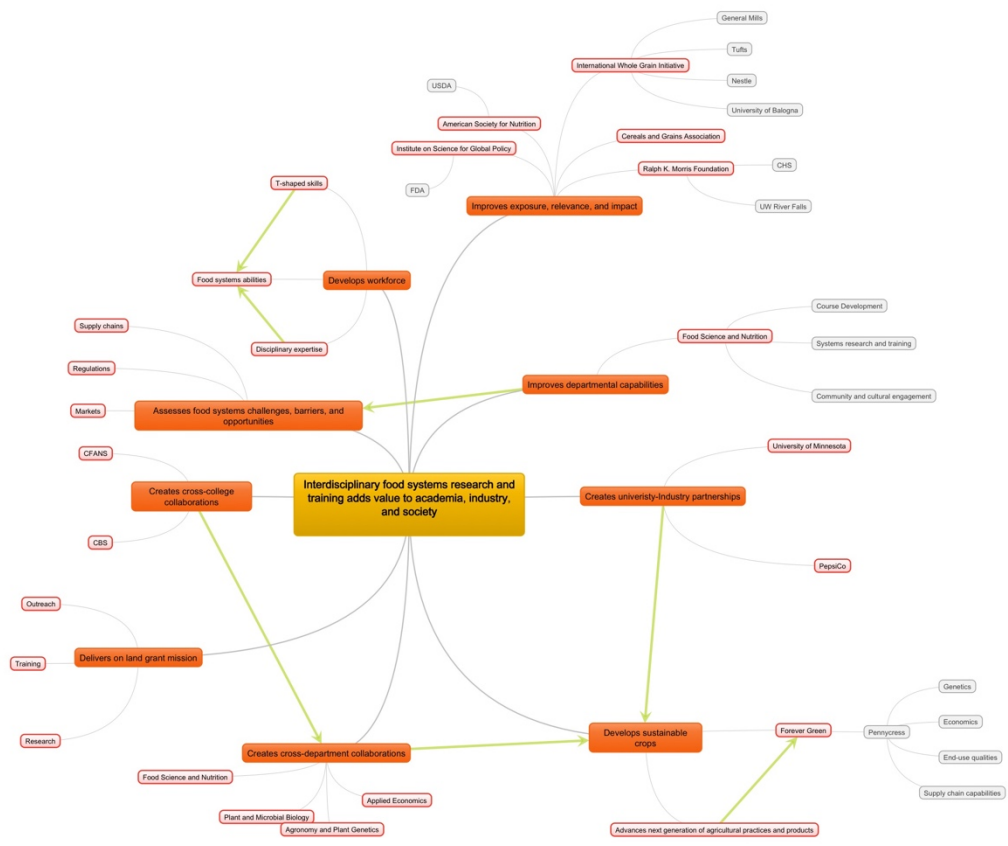


Figure 8.5 illustrates the interlinkages, ongoing projects, and relationships that were generated based on this project.

Epilogue

The research and training described in this dissertation provide a practical example of the integration of cross-disciplinary, cross-sector, cross-supply chain concepts into a nutrition graduate program. Specifically, we aimed to connect agriculture, plant genetics, economics, and food and nutrition sciences to contribute meaningful improvements to the food system. This is most prominently exemplified through our interdisciplinary approach to pennycress development, where we sought to 1) identify genetic targets to improve food qualities and 2) estimate on-farm production economics. We also discussed the role of the university in facilitating sustainable food system development. Finally, we described the components of our training program and offered insights into the value of this integrated framework.

It strikes me that there are a few learnings and benefits of this work that are difficult to describe within the framework of a dissertation chapter. Therefore, I would like to describe, point blank, some personal learnings as well as a few insights regarding the future of the nutrition field in this epilogue.

Personal learnings

As described in chapter 7, this program helped me to conceptualize food systems, identify critical development points throughout the system, and collaborate across disciplines and sectors to address food systems challenges. Developing these abilities ultimately served to recommend the three-step process provided in chapter 8: map the system to understand the issue; identify, analyze and engage stakeholders to prioritize the

critical development opportunity; create and execute a technical strategy to address the problem. The T-shaped skills highlighted in chapter 7 (e.g. critical thinking, cross-disciplinary collaboration, communication, cross-cultural engagement etc.) were essential for these steps. From my perspective, these skills and abilities are likely helpful when dealing with any complex, systems-based problem.

Throughout these experiences, my advisor would often challenge me by asking, “How do you deal with what you do not know?” The answer is simple in theory and extremely difficult in practice: admit to yourself that you do not know, find people who do know, improve your understanding of the issue, and work with those people toward a solution that accommodates all parties. This process requires humility, integrity, accountability, open-mindedness, and flexibility. It is also essential to become comfortable working in the unknown and in ambiguous situations. While I have not always exemplified these qualities, I know that I have grown, and I aspire to attain them.

As cliché as it may sound, understanding “why” is critical to the success of a project. I was fortunate to work on projects that aligned with my personal values and beliefs. I fully believe that developing sustainable food crops can improve the health of our planet and population. Researching and training in this topic area allowed my work to have further purpose, value, and direction. I will take these values into the workforce, pass on what I have learned, and continue to strive for a more sustainable, healthful food system.

Insights regarding nutrition training at the university

The field of nutrition may benefit from pursuing more systems-based approaches. Nutrition ecology may provide a framework to better address the nutritional, environmental, economic, and societal implications of food production and consumption. Our work catalyzed systems-based projects at the University of Minnesota through the creation of a course (e.g. The Intersection of Food Aging and Health), the creation of a cohort conducting systems research in the cultural context of the Midtown Global Market, and the creation of a student group (Student Ambassadors of Food, Culture, and Health), which supplements nutrition and food science curricula with systems learning programming. Each of these programs continues to impact the student body and, in some cases, the larger Minnesota community.

Our work also catalyzed discussions between the College of Food Agriculture and Natural Resource Sciences, the College of Biological Sciences, the Department of Food Science and Nutrition, the Department of Agronomy and Plant Genetics, the Department of Plant and Microbial Biology, and the Department of Applied Economics regarding next generation-training approaches around new crop development. Additionally, the university paper (chapter 6) is currently under review by the President of the University of Minnesota. These discussions in particular may catalyze future integrated, interdisciplinary food systems approaches, which could position the University of Minnesota on the cutting edge of world-class training. This is an admirable goal, and I hope the university seriously pursues this opportunity. The university is capable of achieving this goal, yet it will require alignment of university, college, and departmental infrastructure to implement this training at scale. It may require restructuring incentive

systems (e.g. the tenure process, annual reviews, etc.), and it will require open-mindedness from administration, faculty, staff, and students. Our interdisciplinary approach received a range of institutional support, first being told it was impossible then receiving lukewarm acceptance and culminating in broader acceptance. This is understandable as our model was essentially a pilot program. It is my ultimate hope that others will see this body of work and understand that it is possible to implement systems-based interdisciplinary training approaches. These approaches are essential as we face food systems challenges in the 21st century. After all, as food systems challenges become increasingly complex and interlinked, it stands to reason that we ought to train our future workforce in complex interdisciplinary environments.

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Appendix

Appendix 1 – Supplementary information

Supplementary File 1: Protein sequence alignment of the candidate genes of glucosinolate biosynthesis used in this study for genetic characterization.

MYC3* protein sequence alignment of *Arabidopsis thaliana*, wild-type and mutants of *Thalspi arvensis

```
Ta-myc3-2          --
MNDYFLNQSTATDDNASAAAMEAFIGTNHSTLWPQPSLPPPPPLSQFNEDTLQORLQAL
Ta-myc3-3          --
MNDYFLNQSTATDDNASAAAMEAFIGTNHSTLWPQPSLPPPPPLSQFNEDTLQORLQAL
Ta-MYC3            --
MNDYFLNQSTATDDNASAAAMEAFIGTNHSTLWPQPSLPPPPPLSQFNEDTLQORLQAL
Ta-myc3-1          --
MNDYFLNQSTATDDNASAVMEAFIGTNHSTLWPQPSLPPPPPLSQFNEDTLQORLQAL
At-MYC3            MNGTTSSINFLTSDDDDASAAAMEAFIGTNHHSSLFPP-
PPQPPQPQFNEDTLQORLQAL

Ta-myc3-2          IESAGEG*-----
-----
Ta-myc3-3
      IESAGEGWTYAIFWQISHDFDSSTGDNTVILGWGDGYKGEEDKEKKKNSSSSNSAEQEH
Ta-MYC3
      IESAGEGWTYAIFWQISHDFDSSTGDNTVILGWGDGYKGEEDKEKKKNSSSSNSAEQEH
Ta-myc3-1
      IESAGEGWTYAIFWQISHDFDSSTGDNTVILGWGDGYKGEEDKEKKKNSSSSNSAEQEH
At-MYC3            IESAGENWTYAIFWQISHDFDSSTGDNTVILGWGDGYKGEEDKEKKKNNTN-
-TAEQEH

Ta-myc3-2          -----
-----
Ta-myc3-3
      RKRVIRELNLSISGGTEVSDSNDEEVTDTTEWFFLVSMQSFMNGVGLPGESYLNSRVIW
Ta-MYC3
      RKRVIRELNLSISGGTGVSDSNDEEVTDTTEWFFLVSMQSFMNGVGLPGESYLNSRVIW
Ta-myc3-1
      RKRVIRELNLSISGGTGVSDSNDEEVTDTTEWFFLVSMQSFMNGVGLPGESYLNSRVIW
At-MYC3
      RKRVIRELNLSISGGIGVSDSNDEEVTDTTEWFFLVSMQSFVNGVGLPGESFLNSRVIW

Ta-myc3-2          -----
-----
Ta-myc3-3
      LSGPGALIGSGCERAGQGQIYGLQTMVCIAAENGVELGSSEVLSSHSSDLMDKVNSLFNS
Ta-MYC3
      LSGPGALIGSGCERAGQGQIYGLQTMVCIAAENGVELGSSEVLSSHSSDLMDKVNSLFNS
```

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Ta-myc3-1
    LSGPGALIGSGCERAGQGQIYGLQTMVCIAAENGVELGSSEVLSSHSSDLMDKVNSLFNS
At-MYC3
    LSGSGALTGSGCERAGQGQIYGLKTMVCIATQNGVVELGSSEVISQSSDLMHKVNNLFNF

Ta-myc3-2      -----
-----
Ta-myc3-3
    NNGNGEASSWGFNLNPDQGENDPALWISEPTTTGIESGQVIPAINNSNSNSNSKSDSHQI
Ta-MYC3
    NNGNGEASSWGFNLNPDQGENDPALWISEPTTTGIESGQVIPAINNSNSNSNSKSDSHQI
Ta-myc3-1
    NNGNGEASSWGFNLNPDQGENDPALWISEPTTTGIESGQVIPAINNSNSNSNSKSDSHQI
At-MYC3
    NNGGGNNGVEASSWGFNLNPDQGENDPALWISEPTNTGIESPARVNNNGNSNSNSKSDSH

Ta-myc3-2      -----
-----
Ta-myc3-3
    SKLEKNESSIENPRQQQNPSLVERDLNFSSSGLNQNGNFQDGSSRMMKSNETLSFTAEEES
Ta-MYC3
    SKLEKNESSIENPRQQQNPSLVERDLNFSSSGLNQNGNFQDGSSRMMKSNETLSFTAEEES
Ta-myc3-1
    SKLEKNESSIENPRQQQNPSLVERDLNFSSSGLNQNGNFQDGSSRMMKSNETLSFTAEEES
At-MYC3
    QISKLEKNDISSVENQNRQSSCLVEKDLTFQGG-----
LLKSNETLSFCGNE

Ta-myc3-2      -----
-----
Ta-myc3-3
    NKRRSPVSKGSNNDEGMLSFSSTVVRSAAKSVSDSDHSDLEASVVKEAIVVEPEKKPRKRGR
Ta-MYC3
    NKRRSPVSKGSNNDEGMLSFSSTVVRSAAKSVSDSDHSDLEASVVKEAIVVEPEKKPRKRGR
Ta-myc3-1
    NKRRSPVSKGSNNDEGMLSFSSTVVRSAAKSVSDSDHSDLEASVVKEAIVVEPEKKPRKRGR
At-MYC3
    SSKKRTSVSKGSNNDEGMLSFSSTVVRSAANDSDHSDLEASVVKEAIVVEPEKKPRKRGR

Ta-myc3-2      -----
-----
Ta-myc3-3
    KPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGDAISYINELKSKL
Ta-MYC3
    KPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGDAISYINELKSKL
Ta-myc3-1
    KPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGDAISYINELKSKL
At-MYC3
    KPANGREEPLNHVEAERQRREKLNQRFYSLRAVVPNVSKMDKASLLGDAISYINELKSKL

```

```

Ta-myc3-2      -----
-----
Ta-myc3-3
      QQAESDKEEIQKQLDGMSKEGNREGGGGTKAKERKCSNQDSASSIEMEIDVKIIG-WDVM
Ta-MYC3
      QQAESDKEEIQKQLDGMSKEGNREGGGGTKAKERKCSNQDSASSIEMEIDVKIIG-WDVM
Ta-myc3-1
      QQAESDKEEIQKQLDGMSKEGNREGGGGTKAKERKCSNQDSASSIEMEIDVKIIG-WDVM
At-MYC3
      QQAESDKEEIQKLDGMSKEGNNGKCGSRAKERKSSNQDSTASSIEMEIDVKIIGWDVM

Ta-myc3-2      -----
-----
Ta-myc3-3
      IRVQCSKKNHPGARFMEALKELDLEVNHASLSVVNDLMIQQATVKMGSQFFNHDQLKVAL
Ta-MYC3
      IRVQCSKKNHPGARFMEALKELDLEVNHASLSVVNDLMIQQATVKMGSQFFNHDQLKVAL
Ta-myc3-1
      IRVQCSKKNHPGARFMEALKELDLEVNHASLSVVNDLMIQQATVKMGSQFFNHDQLKVAL
At-MYC3
      IRVQCGKKDHPGARFMEALKELDLEVNHASLSVVNDLMIQQATVKMGSQFFNHDQLKVAL

Ta-myc3-2      -----
Ta-myc3-3      MSKVGEDN
Ta-MYC3        MSKVGEDN
Ta-myc3-1      MSKVGEDN
At-MYC3        MTKVGENY

```

CYP83A1 protein sequence alignment of *Arabidopsis thaliana*, wild-type and mutants of *Thalspi arvense*

Ta-cyp83a1-3
MEDI IIGVVALAAVLLFFLYQSPKTKRYKLPPGPRPLPVIGNLHQLSQVNPQRFFYGWAK
Ta-cyp83a1-2
MEDI IIGVVALAAVLLFFLYQSPKTKRYKLPPGPRPLPVIGNLHQLSQVNPQRFFYGWAK
Ta-CYP83A1
MEDI IIGVVALAAVLLFFLYQSPKTKRYKLPPGPRPLPVIGNLHQLSQVNPQRFFYGWAK
Ta-cyp83a1-1
MEDI IIGVVALAAV**FL**FFLYQSPKTKRYKLPPGPRPLPVIGNLHQLSQVNPQRFFYGWAK
At-CYP83A1
MEDI IIGVVALAAVLLFFLYQKPKTKRYKLPPGPSPLPVIGNLLQLQKLNQQRFFAGWAK

Ta-cyp83a1-3
KYGPILSYKIGNKTMMVISSAELTKELLKTQDVNFANRPPHRGHELMTYGRSDMAMNHYT
Ta-cyp83a1-2
KYGPILSYKIGNKTMMVISSAELTKELLKTQDVNFANRPPHRGHELMTYGRSDMAMNHYT
Ta-CYP83A1
KYGPILSYKIGNKTMMVISSAELTKELLKTQDVNFANRPPHRGHELMTYGRSDMAMNHYT
Ta-cyp83a1-1
KYGPILSYKIGNKTMMVISSAELTKELLKTQDVNFANRPPHRGHELMTYGRSDMAMNHYT
At-CYP83A1
KYGPILSYRIGSRTMVVISSAELAKELLKTQDVNFADRPPHRGHEFISYGRRDMALNHYT

Ta-cyp83a1-3
PLYREMRKMGMNHLFSPTRVATFKHVREEEARRMMFKIEKAAERSEPVDISELMLTFTNS
Ta-cyp83a1-2
PLYREMRKMGMNHLFSPTRVATFKHVREEEARRMMFKIEKAAERSEPVDISELMLTFTNS
Ta-CYP83A1
PLYREMRKMGMNHLFSPTRVATFKHVREEEARRMMFKIEKAAERSEPVDISELMLTFTNS
Ta-cyp83a1-1
PLYREMRKMGMNHLFSPTRVATFKHVREEEARRMMFKIEKAAERSEPVDISELMLTFTNS
At-CYP83A1
PYYREIRKMGMNHLFSPTRVATFKHVREEEARRMMDKINKAADKSEVVVDISELMLTFTNS

Ta-cyp83a1-3
VVCRAQAFGKKYNEDGEEMKRFIRILYGTQSVLGKIFFSDFFPFTRYVLDNWTGLTKYMMD
Ta-cyp83a1-2
VVCRAQAFGKKYNEDGEEMKRFIRILYGTQSVLGKIFFSDFFPFTRYVLDNWTGLTKYMMD
Ta-CYP83A1
VVCRAQAFGKKYNEDGEEMKRFIRILYGTQSVLGKIFFSDFFPFTRYVLDNWTGLTKYMMD
Ta-cyp83a1-1
VVCRAQAFGKKYNEDGEEMKRFIRILYGTQSVLGKIFFSDFFPFTRYVLDNWTGLTKYMMD
At-CYP83A1 VVCRAQAFGKKYNEDGEEMKRFIKILYGTQSVLGKIFFSDFFPYCG-
FLDDL SGLTAYMKE

Ta-cyp83a1-3

CFERQDTYIQEIIIDETLDPNKVKPETESMIDLLMEVYKEQPFASKFTIGNVKGVILNIVV
Ta-cyp83a1-2 CFER*-----

Ta-CYP83A1
CFERQDTYIQEIIIDETLDPNKVKPETESMIDLLMEVYKEQPFASKFTIGNVKGVILNIVV
Ta-cyp83a1-1
CFERQDTYIQEIIIDETLDPNKVKPETESMIDLLMEVYKEQPFASKFTIGNVKGVILNIVV
At-CYP83A1
CFERQDTYIQEVVNETLDPKRVKPETESMIDLLMGIYKEQPFASEFTVDNVKAVILDIVV

Ta-cyp83a1-3
AGTDTAAAAVVWGMTYLMKYPQVMKKAQAEVREYAKEKDLTFITEDDVKNLPHYFRALVKE
Ta-cyp83a1-2 -----

Ta-CYP83A1
AGTDTAAAAVVWGMTYLMKYPQVMKKAQAEVREYAKEKDLTFITEDDVKNLPHYFRALVKE
Ta-cyp83a1-1
AGTDTAAAAVVWGMTYLMKYPQVMKKAQAEVREYAKEKDLTFITEDDVKNLPHYFRALVKE
At-CYP83A1
AGTDTAAAAVVWGMTYLMKYPQVLKKAQAEVREYMKEKGSTFVTEDDVKNLPHYFRALVKE

Ta-cyp83a1-3
TLRIEPIPLLI PRCCI QDTKIAGYDVPAGTTVNVNAWAVSRDEKEWGNPDEFRPR**K**RFL
Ta-cyp83a1-2 -----

Ta-CYP83A1
TLRIEPIPLLI PRCCI QDTKIAGYDVPAGTTVNVNAWAVSRDEKEWGNPDEFRPERFL
Ta-cyp83a1-1
TLRIEPIPLLI PRCCI QDTKIAGYDVPAGTTVNVNAWAVSRDEKEWGNPDEFRPERFL
At-CYP83A1
TLRIEPIPLLI PRACI QDTKIAGYDI PAGTTVNVNAWAVSRDEKEWGNPDEFRPERFL

Ta-cyp83a1-3
EKDVDFKGTDYEFIFPGSGRRMCPGMRLGAAMIEVPYANLLLNFDFKLADGLKPEEINMD
Ta-cyp83a1-2 -----

Ta-CYP83A1
EKDVDFKGTDYEFIFPGSGRRMCPGMRLGAAMIEVPYANLLLNFDFKLADGLKPEEINMD
Ta-cyp83a1-1
EKDVDFKGTDYEFIFPGSGRRMCPGMRLGAAMIEVPYANLLLNFDFKLADGLKPEEINMD
At-CYP83A1
EKEVDFKGTDYEFIFPGSGRRMCPGMRLGAAMLEVPYANLLLSFNFKLPNGMKPDDINMD

Ta-cyp83a1-3 VMTGLAMHKAVHLRLVPEKVRK-
Ta-cyp83a1-2 -----
Ta-CYP83A1 VMTGLAMHKAVHLRLVPEKVRK-
Ta-cyp83a1-1 VMTGLAMHKAVHLRLVPEKVRK-
At-CYP83A1 VMTGLAMHKSQHLKLVPEKVNKY

Supplementary Table 1: Seed glucosinolate values of pennycress lines predicted with NIRS and wet lab analysis

Line Number	NIRS Prediction	Wet Lab Analysis	Source
D0302	42	33	Chopra et al. 2019 - ICP
D0308	42	32	Chopra et al. 2019 - ICP
D0378	37	28	Chopra et al. 2019 - ICP
D0702	70	44	Chopra et al. 2019 - ICP
D0806	67	34	Chopra et al. 2019 - ICP
D0927	70	52	Chopra et al. 2019 - ICP
D0930	70	45	Chopra et al. 2019 - ICP
D0956	66	39	Chopra et al. 2019 - ICP
D3 N13P2	64	62	Chopra et al. 2019 - ICP
d3 n13p4	86	124	Chopra et al. 2019 - ICP
d3 n13p4	86	58	Chopra et al. 2019 - ICP
d3 n13p5	100	76	Chopra et al. 2019 - ICP
d3 n17p3	129	141	Chopra et al. 2019 - ICP
d3 n17p4	39	41	Chopra et al. 2019 - ICP
D3 N44P1	122	99	Chopra et al. 2019 - ICP
D3 N57P16	111	120	Chopra et al. 2019 - ICP
D3 N57P3	60	90	Chopra et al. 2019 - ICP
E0202	69	80	Chopra et al. 2019 - ICP
E0204	69	80	Chopra et al. 2019 - ICP
E0284	67	90	Chopra et al. 2019 - ICP
E0316a	69	78	Chopra et al. 2019 - ICP
E0346	70	80	Chopra et al. 2019 - ICP
E0347	67	82	Chopra et al. 2019 - ICP
E0395	66	94	Chopra et al. 2019 - ICP
E5 035P5	42	75	Chopra et al. 2019 - ICP

E5 035P6	109	102	Chopra et al. 2019 - ICP
E5 068P2	65	72	Chopra et al. 2019 - ICP
E5 068P2	65	54	Chopra et al. 2019 - ICP
E5 068P6	101	76	Chopra et al. 2019 - ICP
E5 069P2	65	87	Chopra et al. 2019 - ICP
E5 069P4	81	79	Chopra et al. 2019 - ICP
E5 069P4	81	72	Chopra et al. 2019 - ICP
E5 070P4	91	96	Chopra et al. 2019 - ICP
E5 070P6	59	72	Chopra et al. 2019 - ICP
E5 106P2	103	72	Chopra et al. 2019 - ICP
E5 106P3	68	69	Chopra et al. 2019 - ICP
E5 156P1	73	82	Chopra et al. 2019 - ICP
E5 156P1	73	78	Chopra et al. 2019 - ICP
E5 156P2	68	62	Chopra et al. 2019 - ICP
E5 156P2	68	61	Chopra et al. 2019 - ICP
E5 166P2	72	70	Chopra et al. 2019 - ICP
E5 166P5	100	94	Chopra et al. 2019 - ICP
E5 167P1	89	72	Chopra et al. 2019 - ICP
E5 167P1	89	71	Chopra et al. 2019 - ICP
E5 167P3	68	74	Chopra et al. 2019 - ICP
E5 186P1	85	91	Chopra et al. 2019 - ICP
E5 186P5	69	90	Chopra et al. 2019 - ICP
E5 188P1	69	68	Chopra et al. 2019 - ICP
E5 188P1	69	68	Chopra et al. 2019 - ICP
E5 188P5	100	114	Chopra et al. 2019 - ICP
E5 189P2	90	116	Chopra et al. 2019 - ICP
E5 189P5	77	82	Chopra et al. 2019 - ICP
E5 189P5	77	80	Chopra et al. 2019 - ICP
E5 189P6	65	69	Chopra et al. 2019 - ICP

E5 198P4	84	81	Chopra et al. 2019 - ICP
E5 198P4	84	80	Chopra et al. 2019 - ICP
E5 199P4	92	95	Chopra et al. 2019 - ICP
E5 199P5	68	79	Chopra et al. 2019 - ICP
E5 199P5	68	79	Chopra et al. 2019 - ICP
E5 202P2	39	48	Chopra et al. 2019 - ICP
E5 202P2	39	44	Chopra et al. 2019 - ICP
E5 202P3	64	86	Chopra et al. 2019 - ICP
E5 247P2	103	94	Chopra et al. 2019 - ICP
e5 294p2	126	112	Chopra et al. 2019 - ICP
e5 294p3	73	70	Chopra et al. 2019 - ICP
e5 296p2	56	34	Chopra et al. 2019 - ICP
e5 296p4	89	84	Chopra et al. 2019 - ICP
E5 337P1	63	66	Chopra et al. 2019 - ICP
E5 337P1	63	65	Chopra et al. 2019 - ICP
E5 337P2	52	65	Chopra et al. 2019 - ICP
E5 339P1	52	76	Chopra et al. 2019 - ICP
E5 359P3	55	67	Chopra et al. 2019 - ICP
E5 359P3	55	58	Chopra et al. 2019 - ICP
E5 367P1	74	78	Chopra et al. 2019 - ICP
E5 367P1	74	73	Chopra et al. 2019 - ICP
E5 367P2	63	91	Chopra et al. 2019 - ICP
E5 444P1	24	20	Chopra et al. 2019 - ICP
E5 484P5	102	91	Chopra et al. 2019 - ICP
F0666c	67	72	Chopra et al. 2019 - ICP
F0808	60	62	Chopra et al. 2019 - ICP
F0823	64	69	Chopra et al. 2019 - ICP
F0900	65	72	Chopra et al. 2019 - ICP
F0903	62	71	Chopra et al. 2019 - ICP

G1126	60	60	Chopra et al. 2019 - ICP
R0141	51	51	Chopra et al. 2019 - ICP
R0355	69	73	Chopra et al. 2019 - ICP
s1	101	111	Chopra et al. 2019 - ICP
s10	59	80	Chopra et al. 2019 - ICP
s11	83	123	Chopra et al. 2019 - ICP
s12	76	94	Chopra et al. 2019 - ICP
s13	52	75	Chopra et al. 2019 - ICP
s14	130	165	Chopra et al. 2019 - ICP
s15	130	172	Chopra et al. 2019 - ICP
s17	94	120	Chopra et al. 2019 - ICP
s18	57	74	Chopra et al. 2019 - ICP
s19	94	108	Chopra et al. 2019 - ICP
s2	83	112	Chopra et al. 2019 - ICP
s20	54	92	Chopra et al. 2019 - ICP
s21	84	109	Chopra et al. 2019 - ICP
s22	100	130	Chopra et al. 2019 - ICP
s23	78	102	Chopra et al. 2019 - ICP
s24	154	180	Chopra et al. 2019 - ICP
s25	135	160	Chopra et al. 2019 - ICP
s26	51	65	Chopra et al. 2019 - ICP
s27	109	144	Chopra et al. 2019 - ICP
s28	60	70	Chopra et al. 2019 - ICP
s29	72	90	Chopra et al. 2019 - ICP
s3	61	112	Chopra et al. 2019 - ICP
s30	55	75	Chopra et al. 2019 - ICP
s31	90	120	Chopra et al. 2019 - ICP
s32	67	100	Chopra et al. 2019 - ICP
s33	42	50	Chopra et al. 2019 - ICP

s34	82	116	Chopra et al. 2019 - ICP
s37	93	134	Chopra et al. 2019 - ICP
s39	123	139	Chopra et al. 2019 - ICP
s4	60	82	Chopra et al. 2019 - ICP
s40	76	117	Chopra et al. 2019 - ICP
s41	76	94	Chopra et al. 2019 - ICP
s42	105	134	Chopra et al. 2019 - ICP
s43	111	146	Chopra et al. 2019 - ICP
s44	97	127	Chopra et al. 2019 - ICP
s45	104	129	Chopra et al. 2019 - ICP
s5	118	146	Chopra et al. 2019 - ICP
s6	55	83	Chopra et al. 2019 - ICP
s7	31	48	Chopra et al. 2019 - ICP
s8	129	161	Chopra et al. 2019 - ICP
s9	106	138	Chopra et al. 2019 - ICP
E3196	-5	9	Chopra et al. 2020 - NF
A7 11	103.3	113.5	Ringling et al. (this study)
A7 137	99.8	104.0	Ringling et al. (this study)
D0262	36	29	Ringling et al. (this study)
D0347	67	86	Ringling et al. (this study)
D3 22	96.6	120.0	Ringling et al. (this study)
E0929	69	52	Ringling et al. (this study)
E1364	69	41	Ringling et al. (this study)
E1825	70	79	Ringling et al. (this study)
E2611	64	82	Ringling et al. (this study)
E5 133P2	57.9	71.2	Ringling et al. (this study)
E5 356P5	92.5	96.7	Ringling et al. (this study)
E5 543	57.9	87.0	Ringling et al. (this study)
E5 301P1	94.5	99.0	Ringling et al. (this study)

MN106 #33	91.2	106.6	Ringling et al. (this study)
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Supplementary Table 2: List of primers used in this study for qPCR analysis and genotyping.

Method	Primer Name	Sequence
qPCR analysis	Actin_Forward	GTGAGACACACCATCACCAGAAT
	Actin_Reverse	TGTCGCCATCCAAGCTGTTCT
	AOP_qPCR_F	GACGGTAACAAGAGCATCAG
	AOP_qPCR_R	AGCCAACAGTAACACCAGCA
	CYP83A1_qPCR_F	ACAAAGAACAACCATTGCGCC
	CYP83A1_qPCR_R	TTCCCGCAACCACTATATTCA
	MAM1_qPCR_F	AAATTCCGGCATTGTTCTTG
	MAM1_qPCR_R	TTTCAGCCGTTCTTTCACAG
Genotyping	cyp83a1-3-common	GGCCCAAACCCTGATGAAT
	cyp83a1-3-allele-x	CGTCCTTCTCAAGAAACCTCTC
	cyp83a1-3-allele-y	CGTCCTTCTCAAGAAACCTCTT
	myc3-2-common	TGAGATCTGCCAGAAAAT

	myc3-2-allele-x	GCAGGAGAAGGCTGG
	myc3-2-allele-y	GCAGGAGAAGGCTGA