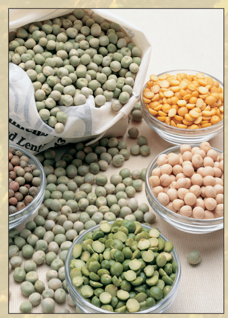
APA
American Pulse Association

Pulse Health Initiative

Strategic Plan

Published September 1, 2010




USA Dry Pea & Lentil Council

APA American Pulse Association

US Dry Bean Council

American Pulse Association

Pulse Health Initiative Strategic Plan

Version 1.3
September 1, 2010

Executive Summary

Pulse Health Initiative Strategic Plan
Executive Summary

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1 Executive Summary

1.1 Introduction

What is a "Pulse" Crop? Pulse crops are defined by the United Nations/Food Aid Organization as dry beans, dry peas, lentils, and chickpeas. Pulses are in the legume family of plants. Legumes are unique among crop plants because they obtain nitrogen from atmospheric sources, rather than requiring extensive fertilizer application of this essential nutrient.

What is the Pulse Health Initiative? The Pulse Health Initiative (PHI) is a focused effort to develop and apply scientific research on the known advantages of pulse crops. Pulses offer outstanding health and nutritional benefits in addition to being good for the environment. The Pulse Health Initiative will deliver science-based solutions to the health, nutrition and sustainability challenges facing the people of this country and the citizens around the world. This Strategic Plan will provide the framework for finding these solutions.

1.1.1 Mission

The mission of the PHI is to provide solutions to the critical health and sustainability challenges facing the citizens of the United States and the global community through research on pulse crops.

1.2 Process and Development of Pulse Health Initiative

PHI Strategic Planning Workshop. On March 30-31, 2010, 52 people representing various sectors of the food industry (i.e., crop production, and scientific research communities familiar with dry beans, dry peas, lentils and chickpeas) met to develop a strategic plan for the PHI. The objective for the planning workshop was to develop short- and long-term research priorities in support of the following three research areas:

- **Health & Nutrition.** Identify ways that the unique attributes of pulses can provide solutions to critical health (i.e. obesity, diabetes, cardiovascular, cancer, etc.) and nutrition issues facing the global population.
- **Functionality/End-Use.** Increase the use of pulse crops through the development of new products or ingredients (using food technologies such as milling, extrusion, extraction (starch, fiber, protein, cooking, etc,) to increase the functionality of these globally acceptable crops.
- **Sustainability.** Identify solutions to the **environmental** (i.e. climate change, nitrogen fixation), **social** (i.e. carbon foot print), and **economic** (i.e. cost/benefit analysis) issues facing the planet, building on the unique attributes of pulse crops.

Workshop Participants and Facilitators: The planning session was facilitated by the following individuals:

- **Health & Nutrition:**
  - Dr. Gerald Combs, Research Leader, USDA-ARS Human Nutrition Center, Grand Forks, ND

- Dr. Michael Grusak, Plant Physiologist, USDA-ARS Children's Nutrition Research Center, Houston, TX.

- **Functionality/End Use:**
  - Dr. Jose de Berrios, Food Science Engineer, USDA-ARS Western Regional Research Center, Albany, CA
  - Dr. Mehmet Tulbek, Technical Director, Northern Crops Institute, Fargo, ND

- **Sustainability:**
  - Dr. George Vandemark, Plant Geneticist and Research Leader, USDA-ARS Grain Legume Research Unit, Pullman, WA
  - Dr. James Kelly, Professor and Plant Breeder, Michigan State University, Lansing, MI.

Participants: A list of participants representing the pulse industry, and a multitude of disciplines and institutions in the scientific community is attached in Appendix 1 of this document.

1.3 Basic Problem Areas of Pulse Industry (Dry Beans, Dry Peas, Lentils and Chickpeas)

1.3.1 Problem Area-Nutrition

While the nutritional benefits and phyto-chemical content of pulse crops are widely recognized, limited evidence exists to establish a clear relationship between intake and chronic disease (obesity, diabetes) reduction (2010 Report of the Dietary Guidelines Advisory Committee). In addition, no mechanistic data exist to establish the dose required to gain the benefits of the pulse crop food components. And, perhaps most importantly, to gain the benefits of these nutrient dense products, populations must consume them. Increased knowledge about health benefits will motivate increases in consumption, but to provide true impacts will require additional investments in functionality and productivity.

1.3.2 Problem Area-Functionality/End Uses

While pulse crops are well recognized for their nutritional benefits, numerous barriers exist that prevent increased consumption. For example:

Health-related constituents of pulses can break down when cooked or processed.

Processing knowledge that will maximize the benefits of pulses, by allowing them to be used as an ingredient in foods does not exist at this time.

Anti-nutritional aspects of Pulse Crops, such as flatulence, or long cooking times, detract from their consumption..

The current knowledge base does not provide enough basic scientific information to reduce the risks associated with developing new pulse-based products. In addition, any increases in demand resulting from developing new functionality for pulse products requires increased productivity.. Lastly, new functionality could require the development of additional traits such as increased protein or improved nutrients that will need to be developed for the industry.

1.3.3 Problem Area-Sustainability

The productivity of Pulse Crops, as measured by average yield, has not increased significantly over the past few decades. World population is expected to increase to 9 billion people by 2050 with the same amount of area or less available for food production. In response, the productivity of pulse crops must increase to meet increased demand for food over the next decade.

While the ability of legumes to fix nitrogen in the soil is widely recognized, this important property is not well documented and is difficult to predict. Furthermore, neither pulse cultivars or Rhizobia bacteria have been developed to specifically increase biological N fixation.

In addition, the carbon and water footprint of pulse production is not well defined and agronomic solutions for enhancing the positive aspects of these footprints have not been developed.

Producers must have a reason to plant pulse crops. With the costs of inputs an ever increasing issue in farming systems across the world, improved Nitrogen fixation, decreased water usage, and improved Carbon sequestration could influence increases in productivity for the industry. Additionally, with the improvements in scientific knowledge provided by nutritional research and functionality efforts, market niches could be provided to increase the profitability of pulses in cropping systems. Globally, improvements in productivity of legumes measured by traditional methods like seed yield and by more complicated characteristics included in sustainability will be very important for global health and food security.

1.4 Strategic Goals of Pulse Health Initiative

1.4.1 Nutrition

- Reduce obesity and associated diseases (Cardio Vascular Disease (CVD), Diabetes, and Cancer) by 50 percent by 2050.
- Reduce global hunger and enhance food security by increasing pulse crop productivity.

1.4.2 Functionality and New Products

- Increase usage of pulses in food products by enhancing their nutritional profile, developing processing knowledge, and reducing barriers to consumption (flatulence, long cooking times.

1.4.3 Sustainability

- Increase productivity of all pulse crops by 30% over the next 20 years.
- Select pulse crops for improved nutrition and health-beneficial traits.
- Reduce carbon footprint of agriculture by increasing the utilization of pulses crops in rotations across the world.
- Reduce water usage for global food production by adding more pulse crops to farming systems.

1.5 Economic Impacts

1.5.1 General Observations –

Providing a detailed estimate of the economic impacts of increased pulse use requires considerable analysis and time. Rather than attempting to provide an oversimplified impact estimate, this report will focus on identifying potential economic impact areas without trying to provide a specific dollar estimate.

1.5.2 Potential Domestic Impacts –

Preliminary research focused on the health benefits of including pulse products in diets indicate measurable benefits for combating obesity, cardiovascular disease, diabetes, and certain cancers. The potential economic impacts of mitigating the effects of these health concerns could be substantial. Some of the direct cost reductions include savings in medical testing and diagnosis, prescription medications and treatment procedures. There are also potential indirect cost savings associated with lower health insurance premiums and reduced employee medical leave time (i.e. fewer missed work days due to illness and/or medical treatment). In addition, there are difficult to measure benefits like improved quality of life for individuals and families that are affected by these medical conditions.

Pulses are a low cost source of dietary fiber, protein and starch. Increased use of pulses and pulse products as food ingredients holds the potential to moderate consumer food costs.

1.5.3 Potential International Impacts –

Many of the economic benefits available to domestic consumers of pulse products are also factors which can influence international customers. For example, obesity, cardiovascular disease, diabetes and cancer are medical conditions that are not unique (or limited) to the United States. Thus, the potential economic benefits realized within the United States can be amplified within the international community.

The expectations for United States agriculture are increasing. Agricultural systems are now expected to provide food, feed, fiber and fuel for a growing world population. However, both the available agricultural land base and water resources are limited. Pulse crops can be an integral component in designing sustainable production systems to effectively utilize limited land and water resources. Pulses are legumes which produce high levels of dietary fiber, protein and starch from each acre while requiring relatively low levels of water (i.e. can be grown in areas with limited rainfall). Adjusting crop rotations and land use to include more pulses has the potential to increase farm level profitability, enhance sustainable agricultural production systems and potentially reduce the damage to environmentally sensitive production regions.

Pulses grown in the United States provide economic benefits to the farmers who grow the crops and the business who condition, process and distribute pulses in both the domestic and international markets. These businesses also provide employment opportunities for people in rural and urban areas of the United States.

1.6 Education and Outreach

- Conduct short courses and training programs for the food industry which will increase the knowledge and awareness for the utilization of pulse and pulse ingredients in food products.
- Conduct short courses, extension and training programs for the end users (school systems, chefs, consumers etc.) and increase the knowledge of the use of pulses in food products.
- Train food industry and consumers on pulse and pulse ingredients as a cost control tool which can assist lowering the costs in retail and commercial food industry.
- Develop/design an active website on Pulse Functionality and End Use that will be able to provide updated information answers to growers, processors, food and restaurant industries, teachers, and the general public.
- Generate active interaction with key representative of the general media (radio, TV, newspapers), scientific and not scientific organizations dealing with food, nutrition, and technology, to promote utilization of pulses as food and ingredients.

1.7 Summary

Pulse crops are an essential part of improving the food delivery system in the world. They are nutritious and environmentally friendly. But, they have been neglected scientifically. This plan for the PHI will begin to use pulses to find solutions to some of the challenges of our world.

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1 Nutrition & Health

1.1 Goals: To exploit the potential of pulse foods to:

1.1.1 Reduce the prevalence of obesity and associated co-morbidities by 50% by 2050.

1.1.2 Reduce global hunger and enhance food/nutritional security.

1.2 Research Priorities:

1.2.1 Identify a market basket of pulses that are representative of global dietary patterns, which can be used to characterize these crops in health-related research.

- ***Understand global dietary consumption patterns***
- ***Characterize types and forms consumed***
- ***Develop market basket based on sound scientific advice***

1.2.1.1 Background

US National survey data show that pulse consumption in the US is relatively low, although estimates vary from study to study. The most recent estimate comes from an analysis of the 2003-2004 National Health and Nutrition Examination Survey (NHANES) (Kimmons et al, 2009). This survey provided 2 non-consecutive days of intake from multiple-pass, 24-hour recalls. Based on that study, 32% of men and 28% of women ≥ 19 years old consumed pulses in any amount over the 2 survey days. In comparison, 35-97% of adults consumed dark green, orange, starchy and other types of vegetables. Therefore, pulses were consumed by the fewest individuals compared to any other group of vegetables. Still, those estimates are greater than those from other studies. Another research group, using data from the 1999-2002 NHANES (Mitchell et al, 2009), reported that ,over a 1 day survey period, 8% of US adults over age 18 consumed pulses. However, a calculation based on the same data indicates that the percentage of US adults consuming pulses may be closer to 20%. In another analysis of NHANES data (Papanikolaou et al, 2008), 18% of adults ≥ 20 years old consumed some form of beans. Two factors may account for the differences in these are estimates: i) whether or not soy beans were included (as in the studies of Kimmons et al [2009] and, possibly, Papanikolau et al [2008]); and ii) the use of different obervation periods (only Kimmons et al [2009] o used a 2-day period). .

Mitchell et al (2009) found the most frequently consumed sources of pulse foods to be pinto beans, refried beans (usually from pintos), baked beans, chili, and Mexican or Hispanic dishes. Other pulse foods (lentils, chickpeas, pink beans, pigeon peas, split peas, fava beans) were also consumed; however none contributed more than 1% to total US pulse consumption.

Even individuals who consumed pulses did so in amounts appreciably less (40-45%) than the recommended ~3 cups per week (Kimmons et al. 2009). In fact, that is likely to be an overestimate of actual pulse consumption, as soyfoods were included in that analysis. Using an estimate of 92 g per half-cup of pulses, an intake of about 79 g/d would be needed to meet the recommended intake. According to the quartile analysis by Mitchell et al. (2009), about half of Americans consume this amount, which is close to the median estimate provided by Kimmons et al (2009). The analysis by Mitchell et al indicates that 66% of adult pulse consumers are typically aged 31-70 years, non-Hispanic white, and have more than a high school education.

Pulses are consumed by people in all regions of the world (Leterne and Muñoz, 2002; Schneider, 2002; Broughton et al, 2003), constituting a major staple in the diets of certain populations. Daily intakes range from 3.5 kg/capita/year in parts of Europe, to 10 kg/capita/year in Latin America, India, or Central Africa, to as high as 40 kg/capita/year in Burundi (FAO, 2009). Nevertheless, pulse foods account for only 2.1% of global energy consumption (cereals account for nearly 50%) (FAO, 2009). Thus, there is ample room for pulse consumption to be increased in all sectors.

1.2.1.2 Research Need/Problem Statement:

The data summarize above indicate there a substantial proportion of the US population can be targeted to consume pulses, and that for at least half of them, pulse intake needs to increase beyond current amounts in order to meet the myPyramid levels of recommended intake from to help achieve a balanced and healthful diet. Globally, the good news is that pulses are an accepted part of the diets of many peoples. It is clear that that more understanding is needed as to how pulses are used in various diets and what factors will lead to their expanded use.

1.2.1.3 Approach Outline of Research Direction:

- Determine barriers to consumption in different sub-populations.
- Determine barriers to meeting the USDA “myPyramid” guidelines for consumption.
- Determine the effect of nutrition education around pulses on pulse consumption, including formal nutrition education and public health messages.
- Determine the effect of making pulses more widely available in prepared foods (e.g., at restaurants and marketplaces).
- Determine the effect on including simple pulse recipes on pulse cans.
- Determine the effect of including pulses in Federal Food and Nutrition Service (FNS) programs such as Women, Infants and Children (WIC), Supplemental Nutrition Assistance Program (SNAP-formerly Food Stamp Program), and School Breakfast and Lunch Programs.

1.2.1.4 Outcomes:

1.2.1.4.1 Immediate (2 years)-

- Increased supermarket sales.
- Increased restaurant sales.
- Increased restaurant menu items.

1.2.1.4.2 Midterm (6 years)

- The above plus US survey data.

1.2.1.4.3 Long Term (10 years)-

- The above plus US survey data.

1.2.1.5 Impacts

1.2.1.5.1 Economical –

- Production and domestic sales of American pulse industry.

1.2.1.5.2 Environmental/Sociological

- Sustainability of consumer markets.

1.2.1.6 Estimated Cost of Research

- We anticipate approximately $5 M per year will be required to realize the short, medium and long-term goals of this project.

1.2.1.7 Bibliography

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1.2.1.8 Relationship Flow Chart

Some health outcomes may also be measured in these types of studies.

1.2.2 Evaluate the effect of the market basket (pulse based diets) on obesity and associated co-morbidities (CVD, cancer and type2-diabetes).

- ***Review existing literature and determine gaps.***
- ***Dose and tolerance, escalation plan.***
- ***Perform coordinated clinical trials.***

1.2.2.1 Need for Well Designed Human Studies

The development of a health platform for pulses will require credible science as a basis for saying that eating pulse foods is good for one's health. Consumers recognize the need for healthier diets, particularly in areas relating to health values of fiber and non-digestible starches. US health (label) claims must be *evidence-based*, i.e., supported by science that is methodologically robust, consistent and replicable. This means that policy-relevant data must come from well designed human intervention studies, i.e. *the science must be credible*.

1.2.2.2 Strengthening the Evidence Base for Pulses

The status of the scientific evidence for health effects of pulses is almost exclusively based on studies with the common bean (*Phaseolus vulgaris*). Strengthening this evidence base will demand **well controlled studies with humans**. The strongest evidence comes from intervention trials that measure effects on validated modifiable risk factors for the incidence of disease. Studies with animal models, while useful in elucidating biological mechanisms, will *not* yield the type of evidence that will advance the goal of promoting pulses on a health platform. This calls for a substantially greater level of investment than is currently being made for human nutrition research.

The strongest cases to be made for specific health effects of pulses are in the following areas, each of which has demonstrable research needs:

1.2.2.3 Weight Management/Obesity Prevention.

Research in several areas is required:

- ***Satiety*** - Human studies documenting the satiating effects of consuming pulses – this would be useful in encouraging inclusion of pulse-based foods in weight management diets.
- ***Inflammation*** – Human studies are needed to determine whether eating pulse foods can reduce inflammatory stress (i.e., pro-inflammatory cytokine levels) in pre-diabetic individuals.
- ***Glycemic Control*** – The apparently low glycemic index of pulses may also have value in promoting these foods on a health platform. However, well designed human studies are needed to characterize the effects of eating pulses on short-term (postprandial) glycemic control. With a few very tightly controlled human studies involving assessment of the postprandial glucose and insulin responses to pulse foods in comparison to other foods, it should be possible to make an evidence-based statement that pulses are a low glycemic index food useful in the short-term regulation of serum glucose, etc. In order to have relevance to diabetes risk, such studies must use fasting blood glucose and insulin sensitivity, the only biomarkers validated as modifiable risk factors for type 2 diabetes.
- ***Starch Blocking*** – Well designed human studies are needed to determine whether the efficacy and safety of the α-amylase inhibitor in beans and/or bean extracts in reducing postprandial hyperglycemia and insulin levels**.**

1.2.2.4 Cardiovascular Health.

Well designed human studies are needed to characterize the effects of eating pulse foods on risk factors for total- and LDL-cholesterol levels. With a few additional, well-controlled human studies, the evidence base is likely to support a qualified health claim at a moderate level, given the apparent magnitude of these effects. Such studies must use the biomarkers have been validated as modifiable risk factors for cardiovascular disease: total cholesterol, LDL-cholesterol, blood pressure.

1.2.2.5 Colon Health.

Well designed human studies are needed to characterize the effects of eating pulse foods on risk factors for colorectal cancer.

- ***Pre-biotic Effects*** – Human studies are needed to determine the impact of eating pulse foods on the colonic microflora which can be assessed in stool specimens using molecular markers. Can pulses be called a "natural pre-biotic"? Can eating pulse foods provide benefits of the type observed for inulin (e.g., enhanced utilization of calcium and other minerals)? In order to have relevance to colorectal cancer risk, such studies must use colonic polyps, the only biomarkers validated as modifiable risk factors for colorectal cancer.
- ***Colonic Adaptation*** – Human studies are needed to determine whether flatulence subsides with the regular consumption of pulses, i.e., whether the hindgut microflora can adapt with respect to gas production and, in this regard, to determine the effects of time, amount and type of pulse consumption.

1.2.2.6 Composition.

Pulses are low-fat, medium-energy foods that are sources of several key nutrients (e.g., protein, folate, calcium, iron, zinc, and magnesium), other bioactive factors (e.g., isoflavones), resistant starch and dietary fiber. However, pulses also contain inhibitors of the amylase required for starch digestion and the folyl "conjugase" required for the absorption of folate. Research is needed to determine both the prevalence of these anti-nutrients, and their respective cooking stabilities/instabilities among the classes of pulses.

1.2.3 Elucidate the underlying mechanisms of the health benefits linked to pulse consumption.

- ***To identify biomarkers and cell signaling pathways (employ "omics" technologies) .***
- ***Conduct mechanistic studies using animal and cell models.***
- ***Identify the bioactive components and the relevant plant genetic components to enhance the health value of these foods.***
- ***Evaluate the effects of regular pulse consumption in healthy people: satiety, inflammation, glycemic control/starch utilization, Cardiovascular Health, colon health (pre-biotic effects, adaptation). Characterize responder/non-responder populations.***

1.2.3.1 Background:

While the epidemiological evidence is supportive of the role of pulses in preventing chronic diseases, the data linking the consumption of specific pulse crops to human health outcomes is limited. This is particularly true with regard to the prevalence of obesity and the occurrence of its associated co-morbidities namely, type-2 diabetes, cardio and cerebovascular diseases, and cancer. It is critical that the current knowledge base be augmented with mechanistic studies that define the bioactive components of pulse crops and the cellular and molecular mechanisms that account for health benefits. Such information is critical for efforts:

1) to improve pulse crops for human health characteristics through contemporary approaches to plant breeding and selection,
2) to increase the consumption of pulses *per se* and of bioactively enriched new food products derived from pulse crops,
3) to engage the public health community in efforts to increase pulse consumption to reduce chronic disease risk, and
4) to develop programs of dissemination intended to improve human health while addressing issues of food safety and security in an ecologically sustainable manner.

1.2.3.2 Research Need/Problem Statement:

In any context, health care providers will demand the following evidence-based facts

1) what pulse crops should be consumed for which diseases,
2) the amount of specific pulses that should be consumed on a daily basis, i.e. the dose,
3) what are the real and perceived barriers to increasing intake,
4) what are the potential adverse effects of increased pulse consumption, and
5) what is the time frame over which pulse consumption must be maintained for human health benefits to be observed.

Strong, scientifically-based answers to these questions cannot currently be provided. Hence, a program of research is required to move this field forward.

1.2.3.3 Approach Outline of Research Direction:

To address deficits in our current understanding of how pulse crops prevent chronic diseases such as obesity and promote human health, a tiered program of mechanistic research is proposed and summarized in four specific aims. They are:

1) *Conduct mechanistic studies using cell-based and animal models* A tiered approach is proposed for pulse crop screening in order to identify which pulse crops and the varieties of each crop therein that have maximal anti-obesogenic activity. It is likely the mouse based models of obesity will provide the greatest insights that translate to the human population with the ability to render information in a timely and cost effective manner. Cell-based models can complement animal studies in validating the signaling pathways affected.
2) *Identify the bioactive components and the relevant plant genetic components to enhance the health value of these foods.* Both primary metabolites of pulse crops (carbohydrate, protein, fat, and nucleic acids) and pulse crop secondary metabolites have been implicated in accounting for human health benefits. Given that unlike other food crops, pulse crops are both high in protein content and rich in their composition of nitrogen-containing secondary metabolites, it is critical to determine how these various components act alone and in combination to account for human health benefits. Such information is critical to efforts directed at crop improvement and for work on the development of new pulse-derived food products.
3) *To identify biomarkers and cell signaling pathways (employ "omics" technologies)* The use of transcriptomics, proteomics, and metabolomics offers an unprecedented opportunity to develop a knowledge base that identifies the fate of pulse components when they are consumed by human populations. Such knowledge is essential for a broad range of issues including: 1) determining compliance to consumption of pulse rich diets, 2) understanding the signaling pathways affected by pulse consumption particularly as it relates to the issue of pulse related-drug interactions especially as pulse intake is increased in populations being treated for obesity and its related co-morbidities, and 3) optimizing the amount and type of pulse crops recommended for individuals at risk for various chronic diseases.
4) ***Evaluate the health effects of regular pulse consumption.*** Well designed studies of the impact of pulse foods on eating rate, satiety and physiological responses to eating in children and adults. These will determine the effects of including pulse foods on eating rate and the hormonal responses of the gut (grhelin, CCK, GLP-1) and other tissues (insulin, leptin, glucose) that affect metabolic function. In addition, robust randomized controlled clinical trials are needed to that focus on specific areas likely to be benefited by pulse consumption: inflammation, glycemic control/starch utilization, dyslipidemia, colonic microbial balance and adaptation. The results of such studies will provide the evidence base for nutrition/food policy related to the roles of pulse foods in promoting and maintaining health
5) *Characterize responder/non-responder populations* Evidence continues to accumulate that irrespective of the life style intervention and/or drug regimen employed to address the occurrence of obesity and its related chronic diseases that there will be individuals in a population who respond to the intervention and those who do not. Given the low prevalence of pulse consumption in most regions of the world and the effort required to

overcome existing barriers to consumption, it will be critical to develop a metric by which to gauge who is most likely to benefit from increased pulse crop consumption.

1.2.3.4 Outcomes:

1.2.3.4.1 Immediate (2 years):

- Determine if all pulse crops are created equal in their ability to reduce obesity and its related co-morbidities

1.2.3.4.2 Midterm (6 years):

- Identify the bioactive food components and the molecular pathways they target for chronic disease prevention

1.2.3.4.3 Long Term (10 years):

- Elucidate biomarkers of human intake and the segments of the population most likely to benefit from increased pulse crop consumption

1.2.3.5 Impacts

1.2.3.5.1 Economical:

- Reduce health care costs by 10% for each 0.5 cup increase in pulse consumption over a period of 5 years.

1.2.3.5.2 Environmental/Sociological:

- Reduce reliance on meat products to support optimal human health thus addressing issues related to impact of our society's nitrogen use, carbon footprint, and water footprint on the global community.

1.2.3.6 Estimated Cost of research:

- 15M/yr for 10 years: 150M

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1.2.4 Develop effective and sustainable programs (national/international) using pulse foods to prevent obesity and related diseases.

- ***Conduct targeted community based intervention trials focused on children, rural populations, inner city, economically underserved populations***
- ***Evaluate sustainability using cultural and economic metrics***

1.2.4.1 Background

Excess body weight (BMI >25) has tripled over the past 50 y (CDC, 2010). Data from the 2005-06 NHANES survey determined the prevalence of overweight, obese, and extremely obese[1] in 20-70 y adults was 32.7%, 34.3%, and 5.9 %, respectively. There are signs that the upward trend in the number of people that are overweight and obese has slowed or stabilized over the past five years; however, the average weight in each category continues to increase implying that the weight problem continues to get worse. In 2000 the US government set a Healthy People Goal of reducing the prevalence of obesity to 15% by 2010; clearly the goal has not been met since current estimates of obesity in US adults are > 40%. While the prevalence of obesity is similar between men and women, there are significant differences in obesity among black, white and Hispanic women (39%, 22%, 29%, respectively) (Flegal et al, 2010).

The prevalence of excess weight among people <20 y is even more alarming because children with high BMIs most often become obese adults. While the percentage of school age children that are obese has stabilized in the past few years, the current percentage (17%) is unacceptable (Ogden et al, 2010). Similarly, the percentage of obese children < 5 y has stabilized over the past five years, but the current percentage (14.6%) is much too high. Like adults, there are income level and ethnicity disparities in obesity prevalence among children (CDC_b). Obesity is greater in children in low-income families and Table 1 shows the differences among ethnicities.

Table 1. Obesity (BMI > 95th percentile) prevalence in children in 2008[a]

Ethnicity	2-5 y low income[b]	2-5 y	6-11 y	12-19 y
all individuals	14.6	10.4	19.6	18.1
Hispanic individuals	18.5	14.2	25.1	21.7
non-Hispanic white individuals	12.6	6.6	20.5	16.7
non-Hispanic black individuals	11.8	11.5	17.7	19.8

[a]Data are from (Ogden et al, 2010)
[b]Data are from (CDC_b). Percentage for American Indians/Alaska Natives is 21.2%

The total (direct + indirect) annual medical cost attributed to excess body weight for adults is estimated to be >$80 B ($CDC_a$). The economic costs for future generations will be even greater considering the high rate of obesity in children. The lifetime medical expenditure due to obesity for a 20 y old is expected to range from $5,340 – $29,460, with costs increasing in proportion to increase in weight (Barkin et al, 2010). For children born between 1982-1993, the loss in income due to obesity is expected to be $1,000 B (Barkin et al, 2010).

[1] These categories are defined by BMI: overweight: BMI >25 -29.9; obese: BMI >39 -39.9; extremely obese: BMI ≥ 40.

The prevalence of excess weight is not restricted to the U.S. – international health agencies have declared there is a global obesity epidemic. The WHO in 2005 estimated that 1.6 B people 15 y and older were overweight and 400 M were obese (WHO). Moreover, many developing countries have the dual problem of inadequate food consumption for some while some are overweight and obese. For the first time, there are as many overweight and obese people in developing countries as there are hungry people (FAO).

Data from large surveys such as NHANES show that individuals that frequently consume pulse foods maintain a lower body weight than non-consumers. These observations led to the hypothesis that consuming a low glycemic index, high fiber meal, in comparison to consuming a high glycemic index, low fiber meal, provides greater satiation (feeling of fullness and satisfaction), lengthens the time interval before hunger signals are experienced (satiety), and reduces food intake in the next meal. Because pulse foods tend to have low glycemic indices and high fiber contents, several studies have been conducted to determine whether eating pulses reduces caloric intake. While some studies supported that hypothesis, more recent ones have not (Wong et al, 2009; Murty et al, 2010). Given the complex etiology of excess caloric consumption, it is very likely that overweight and obese individuals do not stop eating upon satiation and they eat again before true physiologic hunger sets in. However, it also clear that given proper motivation, a diet with a glycemic index <42 and glycemic load <100g will allow weight reduction in overweight/obese individuals and maintenance of desired weight in individuals with BMI < 25 (Livesey et al, 2008). The observed association of lower weight among frequent consumers of pulse foods would appear to be related to their motivation to maintain a healthier diet and body weight.

1.2.4.2 Research Need/Problem Statement

Although plausible, there is insufficient scientific evidence to support recommending that pulses be an important component of strategies to prevent obesity.

1.2.4.3 Approach and Outline of Plan

To address this lack of information the following activities are proposed:

1. Determine effective strategies for convincing caretakers, food manufacturers, schools, communities, etc. that overweight/obesity needs to be avoided especially in children;
2. Determine effective approaches for incorporating pulse foods into diets of pre-school and school age children and effective strategies to sustain pulse consumption as part of comprehensive measures to prevent overweight/obesity ;
3. Determine effective approaches for incorporating pulse foods into diets of various target groups within the U.S. and the world, and effective strategies to sustain pulse consumption as part of comprehensive measures to prevent overweight/obesity;

Multidisciplinary and multinational approaches will be required to achieve these activities. Sustained political commitment from many countries and collaboration among private and public sectors will be required to halt the global problem of obesity. It is critical that impact evaluation utilizing cultural and economic metrics be an integral part of designing and implementation of the activities.

1.2.4.4 Expected Outcomes

1.2.4.4.1 Immediate (2 years):

Develop effective strategies for convincing stakeholders that overweight/obesity in children needs to be avoided.

1.2.4.4.2 Midterm (5 years):

Develop effective approaches to incorporate pulses into diets of target populations as part of a comprehensive approach to prevent overweight/obesity.

1.2.4.4.3 Long term (10 years):

Determine which strategies and approaches are most likely to be sustainable for various sectors of societies and nations.

1.2.4.5 Impacts

- Improved quality of life for 600 M people.
- Improved lifetime workforce productivity in U.S. by $9 T.
- Reduced annual medical costs in U.S. by $50 B due to reduced occurrence of obesity and co-morbidities.

1.2.4.6 Estimated Cost of Research

$5 M per year for 10 years – $50 million total.

1.2.4.7 Bibliography

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2 Functionality & End Use

2.1 Overview of Functionality/End Use

2.1.1 Background:

Pulses, such as dry beans, peas, lentils, and chickpeas, are main legume crops used as sources of human food around the world. They occupy two places on the USDA Food Pyramid-Dietary Guidelines: in both the high-protein and the vitamin-rich vegetable groups. Their consumption is also encouraged by Canada's Food Guide to Healthy Eating, as they are a rich source of quality protein, complex carbohydrates, resistant starch, soluble and insoluble dietary fibers, minerals and B vitamins. They are gluten-free, non-allergenic, and relatively low in fat and sodium. Additionally, pulses are also good source of antioxidants in the form of complex mixtures of phenolic compounds. No database exists with a compilation of nutrient and phytonutrient compositions. The literature has numerous scattered reports on the nutrient composition of various varieties. Fewer reports exist on phytonutrient profiles and these have been obtained on only a few varieties. With no compilation of the compositional data, it is difficult to glean how the contents of nutrients and antioxidative compounds vary between species and between varieties within a species. This lack of knowledge of variation in composition of nutrients and phytonutrients is a barrier to understanding the relationships between functional properties and composition. Changes in pulse composition and functionality during processing or preparation could influence the derived health benefits of pulse food products.

Healthy eating has always been important for proper body growth and development. But more recently it has been accepted that healthy eating is a significant factor in reducing the risk of diet-related health problems including obesity, heart disease, cancer, diabetes, hypertension (high blood pressure), osteoporosis, anemia, and some bowel disorders. The importance of pulses as a valuable source of nutrients is well justified by their excellent nutritional/health value. Therefore, they are a great fit for the healthy eating pattern.

However, in today's retail market there are only few food products that are made from pulses. Moreover, those products are not available as convenient, ready-to-eat foods. This severely limits overall consumption of pulses in USA and hinders their positive health benefiting impact to the general public. Challenges in developing such products include: long cooking time of pulse seeds, flatulence factors, bitter and "beany" flavors, identification of suitable processing technologies to preserve and/or improve the nutritional value and functional properties of pulses or pulse-based food products in a ready-to-eat form, lack of prior knowledge and awareness by general consumers and main stream food processing industry on the value of pulses and how to use them as an important component of the daily diet in USA. Those can be addressed with systematic and concerted innovated research efforts given adequate support and infrastructure.

Concerted efforts of food scientists, cereal chemists, food processing scientists and sensory scientists in basic and applied researches, including the identification of target pulse food products, appropriate raw material selections, introduction of innovative food processing techniques and preparation of convenient ready-to-eat type-foods, will be essential for the successful production, acceptability and marketability of pulse food products.

2.1.2 Strategic Goals Summary for Functionality:

- ***Improve the fundamental understanding of functional properties of pulses and pulse fractions.***
- ***Increase knowledge, awareness and utilization of pulses and pulse ingredients in food products.***
- ***Utilize current, new and innovative technologies to improve pulse and pulse ingredient properties (i.e. reducing cooking time, flatulence factor, and beany flavor in pulses).***
- ***Support Food Security by making available novel pulses, pulse ingredients and pulse-based foods in a convenient and appealing form to serve as carriers of healthy and nutritious components to consumers worldwide.***
- ***Develop strategies for pulses in developing food product solutions which can help in controlling costs in food service and retail products.***

2.1.3 Research Need/Problem Statement:

Development of US pulse market will not reach its potential without the fundamental research to develop a comprehensive compositional database for pulses to understand fundamental physical and chemical properties of pulse components; identify functional and nutritional changes brought about by breeding or processing; characterize composition-function relationships; determine the impacts of processing on composition, nutrition, and functional properties.

In addition, the development of convenient and consumer accepted food products made with pulse and pulse ingredients must be facilitated through innovated research programs in support of reducing obesity/CVD, global hunger/enhance food security, and agriculture's carbon and water footprint. prints. Thus, the research programs are expected be able to:

1) Generate basic information on functional properties of pulses' macro components such as protein, carbohydrate, and resistant starch in raw ingredients and through relevant food processing unit operations.
2) Identify novel food processing technologies, such as pin milling and fractionation, extrusion cooking, infrared radiation, microwave and ohmic heating, size reduction and micro-fluidizing technologies, able to minimize and/or eliminate undesirable components, while preserving and/or improving the nutritional value and functional properties of pulse-based products.
3) Develop novel convenient, tasteful, and appealing food products in a ready-to-eat form; generate scientific information on the use and beneficial consumption of pulses and pulse ingredients to create one success story: a) from farm to fork; b) support nutritional/clinical trials; c) general acceptance by consumers.

2.1.4 Outline of Research Direction:

The objective of this research is to generate fundamental and applied knowledge required to develop healthy and appealing foods form pulses and pulse-based ingredients, which will become a part of our daily meals of the U.S. consumers.

In order to achieve these goals, we need to:

- Conduct a comprehensive evaluation of pulse crop throughout the United States.
- Evaluate functional and nutritional profiles of pulses to identify potential cultivars with greater potential as food.
- Assess the chemical and physical changes that occur in pulse crops due to preparation methods and agronomic practices.
- Develop fundamental knowledge on chemical, functional properties and processing of pulses for the selection of appropriate raw materials.
- Use innovative, short-time & energy efficient food processing technologies for preparation of value-added foods with reduced gas producing oligosaccharides and beany flavor.
- Develop processing solutions for pulses to be used as ingredients in the food industry (i.e. allergen free plant based proteins, high fiber and high dietary fiber ingredients)
- Optimize formulations and processing conditions for preparation of high quality appealing food products, and scale-up the production of pulse foods
- Determine consumer/market acceptance of pulse foods by means of large sensory evaluation tests and surveys for further adjustment of the target food products and improvement of their quality.

2.1.5 Education & Outreach as it pertains to Functionality:

- Conduct short courses and training programs for the food industry which will increase the knowledge and awareness for the utilization of pulse and pulse ingredients in food products.
- Conduct short courses, extension and training programs for the end users (school systems, chefs, consumers etc.) and increase the knowledge of the use of pulses in food products.
- Train food industry and consumers on pulse and pulse ingredients as a cost control tool which can assist lowering the costs in retail and commercial food industry.
- Develop/design an active website on Pulse Functionality and End Use that will be able to provide updated information answers to growers, processors, food and restaurant industries, teachers, and the general public.
- Generate active interaction with key representative of the general media (radio, tv, newspapers), scientific and not scientific organizations dealing with food, nutrition, and technology, to promote utilization of pulses as food and ingredients.

2.1.6 Relationship to PM to ARS/NIFA Priorities as it Pertains to Functionality:

2.1.7 Outcomes by Objective:

2.1.7.1 Impact of Processing on Different Forms of Pulses:

2.1.7.1.1 Immediate (2 years)

- Develop knowledge of impacts of processing on nutritional and chemical properties of pulse components (starch, protein, dietary fiber, polyphenols, vitamins, minerals) and their digestibility. Characterization and understanding structural and functional relationship from farm to fork.
- Develop knowledge of fundamental physical, chemical and nutritional properties of pulses to support health & nutrition and sustainability programs.
- Develop database of information concerning impact of processing on composition, nutrition, and processing properties. Identify and conduct research needed to expand this database.

2.1.7.1.2 Midterm (6 years)

- Develop knowledge on fundamental physical, chemical and nutritional properties of pulses, high protein, high dietary fiber, oligosaccharides, beany flavor, and high amylose pulses.
- Develop technologies to preserve and improve the nutritional value and functional properties of pulses.
- Through research expand databases with further characterization of composition, nutrition, and processing properties.

2.1.7.1.3 Long Term (10 years)

- Develop solutions to improve pulse nutritional and processing qualities in the support of nutrition-health and sustainability programs.

2.1.7.2 Develop Convenient, Healthy, Appealing Products with Pulses:

2.1.7.2.1 Immediate (2 years)

- Develop data bases on nutrients (starch, protein, dietary fiber, vitamins and minerals) and phytonutrients (i.e. polyphenolic compounds) composition; chemical and physical properties, and functionality of pulse seeds, whole flours, and ingredients, from commercial and commercially viable varieties, using existing and new data.
- Identify and/or develop suitable processing technologies to effectively reduce cooking time and eliminate or reduce flatulence factors, beany and bitter flavors on food products made with pulse seeds, whole flours, and/or their ingredients.

- Identify and develop two to three convenient, ready-to-eat, main stream type food products, acceptable to US consumers that can be readily adopted by the food industry for commercial production.
- Develop preliminary engineering and processing data to provide information on energy production needs, water-waste, and scale-up requirements for existing and potential new technologies.

2.1.7.2.2 Midterm (6 years)

- Use preliminary engineering and processing data to reduce energy production needs, water-waste, and optimize scale-up requirements of developed technologies.
- Establish a model system for production of pulse foods with optimized formula and processing conditions.
- Developed two to four general types of convenient food products widely used in school programs and by general public.

2.1.7.2.3 Long Term (10 years)

- Develop production infrastructure including analytical laboratories, pilot-scale processing units, and packaging facilities, unique for pulse processing. Also develop research consortium with close ties to food processing companies and pulses growers.
- Make available healthy, convenient and appealing pulse and pulse-based foods, which can be part of our daily meals.
- Develop data to support the health claims.

2.1.7.3 Physical & Chemical Properties to Support Health/Nutrition and Sustainability Researchers:

2.1.7.3.1 Immediate (2 years)

- The primary outcome of the initial studies will be a complete physical and chemical profile of the current commercial pulse varieties. Preparation of samples to initiate health related studies will be generated.

2.1.7.3.2 Midterm (6 years)

- The primary outcome will be a complete physical and chemical profile of the processed pulses obtained from common preparation methods.

2.1.7.3.3 Long Term (10 years)

- Chemical and physical profiles of samples obtained from newly develop varieties obtained from the sustainability researchers.

2.1.7.4 Pulse Production & Quality to Support Sustainability:

2.1.7.4.1 Immediate (2 years)

- The primary outcome of the study will be to develop the roadmap which will assist the development of physical, nutritional and chemical database profile of the current pulse varieties grown in the U.S. This data will assist breeders to develop varieties based on the needs of growers, processors and end users.

2.1.7.4.2 Midterm (6 years) and Long Term (10 years)

- The outcome of the research will be the development of the enhanced database of the U.S. pulse varieties in terms of physical, chemical and nutritional properties which will assist in enhanced variety selection and development of the breeding programs (i.e. low trypsin inhibitor, low oligosaccharides, high dietary fiber and high protein varieties).

2.1.7.4.3 Long Term (10 years)

- Develop the infrastructure of pulse variety testing laboratories, enhanced analytical testing labs unique for pulse variety evaluation.
- Develop new and enhanced pulse varieties which will support the utilization of pulse and pulse ingredients as a healthy food.

2.1.7.5 Vegetable Proteins:

2.1.7.5.1 Immediate (2 years)

- Identify and develop affordable technologies and methods to fractionate, separate, and concentrate pulse proteins and develop solutions of utilizing protein concentrates and isolates.
- Apply innovative technologies and methods to develop novel and appealing foods (e.g. meat analogs, high protein drinks) utilizing pulses and pulse ingredients as vegetable proteins.

2.1.7.5.2 Midterm (6 years)

- Develop optimized marketing strategies and models for the use of pulse proteins in food products for primarily low income consumers.
- Develop formulations for health based school systems and create a new consortium which will be supported by school systems, consumer groups, pulse processors and food industry.

2.1.7.5.3 Long Term (10 years)

- Develop solutions for the use of pulse proteins in sustainable nutrition-health programs (primarily for children, i.e. snack bars, trail mixes and snack foods) supported by U.S. Government and other possible initiatives.

2.1.7.6 Impacts:

2.1.7.6.1 Economical:

1) Increased knowledge and awareness of the benefits of consuming pulses will increase the market share of pulses, pulse ingredients, and pulse-based foods, which will enhance the health of U.S. and global consumers (cardiovascular diseases, diabetes and allergens).
2) Increased production of food products and ingredients from pulses developed by innovative and energy efficient technologies (i.e. fractionation, extrusion) will provide expanded profits to growers and processors.
3) New value-added products incorporating pulses will have opportunity to compete/share profits with cereals and other foods in the main five Global Market Categories – Savory Snack, Breakfast Cereal, Functional Foods, Gluten-Free, and Beverage/Drink industries.
4) Valuable pulse database for use by growers, processors, and the health industry (nutritionists/clinicians, medical doctors).
5) Develop strategies for pulses in developing food product solutions, which can help in controlling costs in food service and retail products.

2.1.7.6.2 Environmental:

1) Pulses are environmentally friendly crops compared to wheat, rice, corn and soy beans, since they are short cool season crops, requiring less pesticides and water for production.
2) Pulses are a nitrogen fixating crop. Thus, they replenish nitrogen and benefit the environment due to their low carbon footprint.

2.1.7.6.3 Sociological:

1) Create knowledge and awareness on health and nutritional value of pulses and pulse-based foods will ultimately lead to changes to healthier dietary habits.
2) Pulses and pulse-based foods improve the overall health and well-being of consumers by helping to reduce obesity and their associated diseases (e.g. heart disease and diabetes).
3) The improved human health stemming from the increased consumption of pulse foods

2.1.7.7 Estimated Cost of research:

To provide the greatest impact in the shortest amount of time we anticipate a budget need of $5M/year, over the next five years period, for each of the three research groups – Nutrition, Functionality/End Use, and Sustainability.

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3 Sustainability

3.1 Increase productivity through development of improved varieties and sustainable management strategies to improve global nutrition.

3.1.1 Breeding for Increased Yield of Nutritional Health Quality Traits.

3.1.1.1 Background:

In the USA, pulse crops, including peas, lentils, chickpeas and dry beans are cultivated on over 2.935 M acres with an annual production value in excess of $1.1 B. These crops are desirable rotation alternatives to cereals because they break disease and weed cycles, fix nitrogen and sequester carbon, which improves soil fertility and promotes sustainable cropping systems. In addition, cool season legumes are often successfully grown on marginal soils in the absence of irrigation or fertilizer applications, which increases crop options for growers. Pulse crops, including peas, lentils, dry beans and chickpeas currently provide over 12 % of the plant protein consumed by humans globally, more than either potatoes or vegetables (FAO, 2009). Peas, lentils and chickpeas have high protein concentration relative to other sources of plant protein, typically ranging from 16-31% of total seed weight and have especially high levels of the amino acid lysine (Grusak, 2010). Peas, lentils and chickpeas have high protein concentration relative to other sources of plant protein and have especially high levels of the amino acid lysine (Iqbal et al. 2006). A half-cup serving of cooked beans provides ~7g of protein, i.e., ~14% of the recommended daily allowance (RDA). Consumption of bean and cereal in ratios of 1:2 to 1:4 per weight provides all essential amino acids (Bressani, 1981).

Besides their contributions of dietary protein, peas, lentils, and chickpeas also provide essential mineral nutrients to the human diet. Lentils are excellent sources of dietary copper and phosphorus, with a single cooked serving providing over half of the adult US RDA (Grusak 2009). Pulse crops contain all 17 essential elements.

Grusak and Coyne (2009) recently screened 207 lentil accessions from the USDA *Lens* single plant core collection for seed mineral profiles and protein concentration. Concentrations of specific minerals were shown to vary significantly, with the amount of Fe, Zn and Mg varying 4.9 fold, 3.7 fold and 2.5 fold, respectively among accessions. Protein levels in lentils have been shown to vary from 16-31% of total seed weight (Grusak, 2009). Genetic variability for seed Fe concentration in common bean ranges from 34 to >100 mg kg^{-1} and from 21 to 54 mg kg^{-1} for seed Zn concentration (Beebe et al., 2000). Bean breeders at the International Center for Tropical Agriculture (CIAT), in conjunction with Harvest Plus, are working to develop bean lines biofortified with Fe and Zn. They have just released germplasm of the red mottled seed class targeted to production areas in Latin America, and eastern and southern Africa with Fe levels ~20 mg/kg higher and Zn levels 8 mg/kg higher than the recurrent parents (Blair et al., 2010). Biofortified beans targeted to the US market would add value and potentially increase consumption.

Genetic diversity for seed mineral concentration in the 480 accessions of the USDA *Pisum* core collection grown in controlled, non-nutrient limiting conditions has been characterized (Grusak, 2003, data for individual accessions available at http://www.ars-grin.gov/cgi-bin/npgs/html/crop.pl?177). There was a significant range of genetic diversity observed for both the seed macronutrient (K, P, Mg and Ca) concentrations (1.6 fold for Mg to 8.6 fold for Ca) and micronutrient (Fe, Zn, Mn, Cu, B, Mo and Ni) concentrations (3.5 fold for Fe to 6.9 fold for Mn). There is also significant diversity among the accessions of the Pisum core collection for seed protein concentration (Coyne, 2005). The average protein concentration was 20.96%, the range is 12.38 – 30.93.

Differences among varieties for mineral and protein concentrations has also been observed in chickpea. Protein concentration among the kabuli chickpea accessions at ICARDA ranged from 14-27% (Singh et al. 1990).). Seed mineral composition has been determined for 37 kabuli and desi chickpea cultivars grown in a single field location in Spain (Ibanez et al. 1998). Significant differences between cultivars were observed for the concentrations of seed calcium (Ca), Mg, and potassium (K).

3.1.1.2 Research Need/Problem Statement:

Pulse crops, in conjunction with small grains, globally provide the great majority of dietary protein and mineral nutrients to over 2 billion humans who are economically compromised to the extent that they cannot afford to consume significant quantities of animal products in their diet. Micronutrient deficiencies, especially in iron (Fe) and zinc (Zn), are often prevalent where pulse crops are a substantial component of the human diet.

Although pulse crops have levels of seed protein that greatly exceed the amount present in other grains, vegetables and fruits, they are low in the sulfur-containing amino acids methionine and cysteine. With respect to mineral nutrients, although pulse crops possess all 17 minerals that are essential for human diets, they are relatively low in several of these minerals, which must be obtained by other dietary sources. Lentils are extremely low in calcium, contributing only 3% of RDA (in a common serving size), and even this fraction is likely to be unavailable to diet as it is in the form of insoluble calcium oxalate crystals (Franceschi and Nakata, 2005). Lentils are also low in selenium (Se) and magnesium (Mg), providing less than 20% RDA (Grusak 2009). Micronutrient deficiencies, especially in iron (Fe) and zinc (Zn), may be prevalent where pulse crops are a substantial component of the human diet (Gunes et al. 2007).

3.1.1.3 Approach Outline of Research Direction:

The nutritional quality of pulse crops will be improved by increasing the levels of mineral nutrients and proteins in these crops. Research to increase the concentration of minerals and proteins will focus on determining the relative contribution of genetic and environmental factors on these traits. Plant materials will include non-adapted lines and elite breeding lines and varieties of peas, lentils, chickpeas, and dry beans. Plant materials will be grown in replicated trials in multiple environments and harvested seed will be subjected to analysis to determine concentrations of specific minerals and seed protein content. Soil mineral composition will also be determined for each trial location to examine the relationship between soil and seed mineral content. Plant materials will be identified that have the highest levels of minerals and seed protein in specific environments and lines that have stable rankings for these traits over multiple environments will also be identified. Correlations will be determined between the concentrations of various minerals and also between specific mineral concentrations and seed protein content. Crosses will be made between lines that differ in seed protein content and concentrations of specific minerals and populations will be developed from these crosses to determine the inheritance of these traits. This information will provide breeders with guidance for subsequent efforts to develop elite lines with enhanced seed protein content and mineral concentrations.

3.1.1.4 Outcomes:

3.1.1.4.1 Immediate (2 years)

- Plant lines will be evaluated over multiple locations to determine the relative concentrations of specific minerals and seed protein content. Lines with superior levels of minerals and protein will be identified
- The concentrations of specific minerals will be determined for soil samples collected from each trial location.
- Correlations will be determined between the concentrations of various minerals and also between specific mineral concentrations and seed protein content.

3.1.1.4.2 Midterm (6 years)

- The relative importance of genetic and environmental factors on mineral concentrations and seed protein content will be determined.
- Development of new pulse varieties with enhanced nutritional seed characteristics will progress.
- Regions within the US will be identified that can be used to produce pulse crops with enhanced nutritional seed characteristics.

3.1.1.4.3 Long Term (10 years)

- New varieties of pulses with enhanced nutritional seed characteristics will be fully developed and commercially produced.

3.1.1.5 Impacts:

3.1.1.5.1 Economical

We anticipate that the development of pulse crops with enhanced nutritional qualities will increase the annual value of these crops produced in the US by at least 10%. Based on crop values for 2009, this would result in an increase of annual value of approximately $110 M.

3.1.1.5.2 Environmental

We anticipate that the development of pulse crops with enhanced nutritional qualities will increase the acreage of pulse crops produced in the USA. This would result in a reduction in fertilizer, pesticide and herbicide usage in these areas of cultivation, as pulse crops typically require very little inputs of these agrochemicals as compared to other crops. The development of new virus resistant varieties of pea would reduce the amount of insecticide applied annually for controlling vectors of these viruses. The development of new chickpea varieties with resistance to Ascochyta blight and dry bean varieties with resistance to Sclerotinia white mold disease would nearly eliminate the use of fungicides for these crops. This would result in improved soil and water quality and would also reduce health risks to non-target organisms, including humans, associated with exposure to these agrochemicals.

3.1.1.5.3 Sociological

Increased consumption of healthy pulse crops would result in a healthier global population. Increased production of pulse crops in the US will enhance the economic viability of rural communities in which these crops are grown.

3.1.1.6 Estimated Cost of Research

We anticipate approximately $15 M will be required to realize the short, medium and long-term goals of this project.

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3.1.2 Improving Dry Bean Digestibility by genetically reducing the oligosaccharides in the seed

3.1.2.1 Background:

A commonly cited reason for why people don't eat more beans is the gastric discomfort and flatulence associated with eating them. This is a factor in the United States, Europe, and Latin America (Leterme and Munoz, 2002; Schneider, 2002; Winham et al., 2008). Dry beans are especially difficult for infants and young children to digest, thereby limiting their use for this age group to highly processed or fermented products. This is an important limitation because weaning foods containing beans are an excellent tool to combat malnutrition (Rodríguez-Bürger et al., 1998).

The oligosaccharides raffinose and stachyose present in dry seeds are largely responsible for gastric discomfort and flatulence often associated with dry bean consumption (Olson et al., 1981). Raffinose family oligosaccharides also reduce the metabolizable energy of a food (Martinez-Villaluenga et al., 2008). Raffinose and stachyose oligosaccharides make up about three to six percent of the carbohydrate of mature dry beans (Fialho et al., 2006).

Humans do not have the alpha galactosidase enzyme to break the alpha galactoside bonds of oligosaccharides but intestinal microflora do break down the sugars to produce carbon dioxide, hydrogen, nitrogen, oxygen, and methane gas (Olson et al., 1981). Rats fed soy flour with raffinose removed had higher protein digestibility than those fed soy flour with raffinose oligosaccharides (Brasil et al., 2010).

Oligosaccharides can be reduced in beans by 40% with autoclaving or fermentation treatments as compared to cooking via boiling (Khattab and Arntfield, 2009). These sugars have been reduced in navy, black and red beans by 30 to 50% with the addition of the alpha-galactosidase enzyme to finely ground bean flour. The process requires 1 hr at room temperature but is not possible on whole beans, thereby limiting its use (Matella et al., 2005).

Raffinose and stachyose oligosaccharides have been reduced genetically in soybean. Phenotypic screening of EMS mutagenized soybean lines led to the identification of a low raffinose and stachyose line with a mutation in a seed expressed myo-inositol 1-phosphate synthase gene (Hitz et al., 2002). Another low stachyose line was identified for which the candidate gene is a galactosyltransferase. This gene adds a galactose group to sucrose to produce stachyose. (Kerr and Sebastian 2000; Skoneczka et al., 2009).

In legumes, genes involved in the production of raffinose and stachyose sugars have been identified and sequenced including Raffinose synthase (EC 2.4.1.82) in pea (*Pisum sativum*), raffinose synthase and stachyose synthase in soybean (*Glycine max*), and stachyose synthase in adzuki bean (*Vigna angularis*) (Dierking and Bilyeu, 2008; Peterbauer et al., 1999; Peterbauer et al., 2002; Skoneczka et al, 2009). The gene sequence information allowed a reverse genetics approach to identify a mutation in the raffinose synthase (*Rs2*) gene in EMS mutagenized soybean populations (Dierking and Bilyeu, 2009). The line contained 165% sucrose of wild type and 18% of raffinose and 33% of the stachyose. There was no difference in germination between the low RFO and the wild type soybean (Dierking and Bilyeu 2009). A genetic engineering approach has also been used to reduce seed oligosaccharide levels. Overexpression of an α-galactosidase gene from coffee (*Coffea arabica* L.) resulted in pea seed with 40%

reductions in raffinose and stachyose. There was no negative effect on germination rates (Polowick et al., 2009).

3.1.2.2 Research need:

A dry bean with genetically reduced levels of raffinose and stachyose would be valuable to improve consumer acceptance and expand market potential. While there has been research on this area in soybean and pea, there has not been significant research in this area in dry bean.

3.1.2.3 Approach:

EMS mutagenesis: Forward and reverse genetic screens will be used to identify low raffinose/low stachyose beans in chemically mutagenized populations. EMS is a useful mutagen for identifying reduced gene function. EMS causes a high point mutation density (Greene et al., 2003). It is estimated that a plant population of 5000 lines is required to cover the genome (Porch et al., 2009). EMS mutagenized dry bean populations are currently being developed in four commercially important varieties in the Mesoamerican and Andean gene pools and four landraces of the Andean gene pool by USDA-ARS scientists Victor Raboy and Karen Cichy.

Mutant identification: Seed sugar levels will be measured via HPLC. Individual seed will be freeze dried, ground, and weighed. Sugars will be extracted with 80% ethanol, eluted on a Waters SugarPak column and detected with a refractive index detector. While HPLC is the most accurate way to quantify oligosaccharides, long run times (12 min per sample) reduce its efficiency for large sample numbers. For that reason, we will also test a high throughput screen: an enzymatic microplate raffinose assay developed by Karen Fugate (ARS, ND, unpublished)**.**

Reverse genetic screening will be used to screen for single nucleotide polymorphism in the two genes involved in the production of raffinose and stachyose. A modified version of the reverse genetics approach TILLING (targeted induced local lesions in genomics) will be used (McCallum et al., 2000). Sequence information for the raffinose synthase gene and stachyose synthase gene are available in GenBank for soybean will be used to identify gene sequence in dry bean.

Screening for SNPs will be conducted using high resolution melting (Roto-Gene, Qiagen). DNA will be extracted from individual mutagenized plants. Using high resolution melting, homozygote wild type, heterozygotes, and homozygote mutants can all be distinguished. This is very important, especially in cases where the homozygote mutant may be lethal. Lines identified containing SNPs will be evaluated phenotypically for changes in seed sugar levels. Low oligosaccharide lines identified by forward or reverse genetic screens will be backcrossed for ~ 6 generations to the wild type to isolate the low raffinose/stachyose mutations from other mutations caused by the EMS treatment. The effect of the mutation on plant germination, growth, seed yield and seed quality will also be investigated.

3.1.2.4 Outcomes:

3.1.2.4.1 Immediate (2 years)-

- Identification of low raffinose and/or low stachyose bean mutants.

3.1.2.4.2 Midterm (6 years)-

- Information on how the low oligosaccharide trait will impact the agronomic quality and seed quality of the bean. Bean germplasm low in oligosaccharides will be available to processors and other interested parties**.**

3.1.2.4.3 Long Term (10 years)-

- Information on how the low oligosaccharide trait will impact nutritional value of the seed and if it renders the product more useable by consumers.

3.1.2.5 Impacts

3.1.2.5.1 Economical :

Increased use of dry bean will benefit growers, processors, etc.

3.1.2.5.2 Environmental:

neutral.

3.1.2.5.3 Sociological:

The low oligosaccharide bean is intended to address major factor that prevent people from eating more pulses.

3.1.2.6 Estimated Cost of research:

Identification and agronomic testing: $50,000. Animal and human testing: $5 million

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3.1.3 Identify diverse genetic approaches that facilitate development of new pulse crops and varieties.

3.1.3.1 Background:

Plants cultivated under field conditions normally do not realize the maximum possible yields. One method for estimating the magnitude of yield depression is to compare the highest recorded yields with average yields. In the United States during the period up to 1975, average yields of several important crop species were several times less than maximum recorded yields (Boyer, 1982). Record yields were at least 4 times greater than average yields for corn, wheat, soybeans, sorghum, oats and barley. These observations suggest that 1) the genetic potential exists for very high yields under optimal field conditions, and 2) average field conditions are characterized by the presence of factors that adversely affect production.

The development of new plant varieties is a dynamic process that must continually respond to a diverse range of factors that include consumer and market preferences, biotic stresses including diseases and insect pests, and abiotic stresses that include cold, drought, heat, and soil quality. Increased exposure of plants to adverse biotic and abiotic factors is often a consequence of cultivation in marginal environments that are at the limit of the plants' adaptive abilities. Pulse crop production would increase with the development of varieties with enhanced tolerances to adverse abiotic and biotic factors. Classical breeding techniques have only had limited success in the improvement of tolerances to abiotic factors such as drought, cold and salinity. Phenotypes that are influenced by environmental factors are by definition 'quantitative' traits. Genetic determinants for the inheritance of quantitative traits involve multiple loci which exert epistatic, pleotropic and additive effects (Falconer, 1984). Improvement of such traits is often difficult because of linkage between alleles conditioning tolerance to abiotic factors and other alleles that are deleterious to plant performance.

Alternative genetic approaches may be necessary to develop improved pulse varieties in a timely manner to meet future global demands for these crops. One possible avenue of research to meet these breeding objectives is to identify useful genetic markers that can be used to directly screen plants at the seedling stage for desirable traits. A benefit of the use of marker-assisted-selection (MAS) is that the presence of a predictive genetic marker in a plant genome is independent of any confounding environmental factors. This will ensure that a breeder is selecting a plant that exhibits superior performance due to exceptional genetics rather than due to being grown in an environment that favors high performance. In addition, the ability to screen plants for a desirable trait at the seedling stage through the use of a molecular marker will facilitate the efficiency of variety development by allowing breeders to immediately discard plants that do not have the predictive marker, which affords opportunities for reducing costs associated with greenhouse or field space, screening techniques, and personnel. Among pulse crops, the greatest success in MAS has been observed for bean (Miklas et al. 2006), although at least one very useful marker has been developed for identifying pea lines that may have resistance to Aphanomyces root rot (Pilet-Nayal et al. 2005).

Advances in molecular biology and plant transformation have led to the production of transgenic plants that express various foreign genes. The production of transgenic plants can be an efficient method for improving specific phenotypes. If the incorporation of a transgene into a plant genome results in the desired improvement, more gain may be realized in this single 'cycle' of selection than could be obtained in several cycles of traditional plant breeding. A review of the challenges and successes associated with the development of transgenic plants for the improvement of field characteristics limiting crop production suggests that the development of transgenic lines with enhanced tolerance to abiotic factors, such as cold, drought, and trace metal contamination of soil, appears to be a considerable challenge (Vandemark, 1998). The development of transgenic plants has proceeded more rapidly for the enhancement of resistance to insect pests and pathogens. Several transgenic products are commercially available that have resistance to insect pests or viruses (Vandemark, 1998). These efforts have resulted in the production of transgenic species of considerable economic importance, including cotton, squash, rapeseed, tomato and potato. Transgenic varieties with resistance against several classes of plant pathogens may be commercially available in the near future.

3.1.3.2 Research Need/Problem Statement:

Conventional plant breeding is a laborious and time consuming process. The timely development of new pulse crops is a critical component of maintaining and enhancing global food security. New methods need to be developed and applied to accelerate the development of improved pulse varieties. Effective MAS strategies and the application of transgenic technologies are two innovative approaches that will assist in the more timely development of improved pulse varieties.

3.1.3.3 Approach Outline of Research Direction:

Efficient tissue culture and plant transformation protocols will be developed for peas, lentils, chickpeas and dry bean. Methods will be developed to modify the expression of endogenous genes in these crops to realize desired phenotypes including increased yield, disease resistance and tolerance to abiotic stress. Genes from heterologous species will be introduced into these crops and the phenotype of resulting transgenic plants will be determined. Seed from transgenic lines will be increased and lines will be evaluated under field conditions. Efforts will be initiated to obtain regulatory approval for commercialization of these transgenic lines.

Lines of peas, lentils, chickpeas, and dry beans will be identified that differ for important agronomic traits such as yield, disease resistance, and tolerance to abiotic stress. These lines will be crossed to develop populations that segregate for these traits and are useful for mapping. A range of molecular markers, including simple sequence repeats (SSRs), single nucleotide polymorphisms (SNPs) and sequence related amplified polymorphisms (SRAPs) will be developed and used to screen parental lines and segregating progeny. Associations between traits and the presence of specific markers will be identified. The utility of candidate markers for MAS will be evaluated using a range of target plant lines.

3.1.3.4 Outcomes:

3.1.3.4.1 Immediate (2 years)

- Efficient tissue culture and transformation protocols will be developed for pulse crops.
- Crossing strategies will be determined for developing populations that can be used to identify associations between molecular markers and traits of interest.
- Useful marker systems will be developed for improving the utility of MAS in pulse crops.

3.1.3.4.2 Midterm (6 years)

- Transgenic lines of peas, lentils, chickpeas and dry beans will be developed.
- Methods for modifying gene expression in pulse crops will be optimized.
- Associations between traits and the presence of specific markers will be identified.
- Markers that are useful for MAS across a range of environments and diverse genetic backgrounds will be developed.

3.1.3.4.3 Long Term (10 years)

- Transgenic lines of pulse crops with improved disease resistance and abiotic stress trait portfolios will be evaluated under greenhouse and field conditions.
- Environmental impact statements (EIS) for transgenic pulse lines with desirable commercial traits will be completed.
- Useful DNA markers for MAS in pulse crops will be disseminated to regional, national, and international breeding programs.

3.1.3.5 Impacts:

3.1.3.5.1 Economical

We estimate that at least 20% of the annual yield potential of pulse crops grown in the US is lost due to diseases and adverse abiotic stress. Based on crop values for 2009, this would be a loss of approximately $220 million. A considerable portion of these potential losses would be eliminated through the development of new pulse varieties having enhanced resistance to diseases, insect pests and abiotic factors. These new varieties could be developed in a much more timely manner using MAS and transgenic technologies.

3.1.3.5.2 Environmental

We anticipate that the development of pulse crops with enhanced tolerance to diseases and abiotic stress would result in an increase in the acreage of pulse crops produced in the US. This would result in a reduction in fertilizer, pesticide and herbicide usage in these areas of cultivation, as pulse crops typically require very little inputs of these agrochemicals as compared to other crops. The development of new virus resistant varieties of pea would reduce the amount of insecticide applied annually for controlling vectors of these viruses. The development of new chickpea varieties with resistance to Ascochyta blight and dry bean varieties with resistance to Sclerotinia white mold disease would nearly eliminate the use of fungicides for these crops. This would result in improved soil and water quality and would also reduce health risks to non-target organisms, including humans, associated with exposure to these agrochemicals.

3.1.3.5.3 Sociological

The ability to expand the range of areas in the US under production of pulse crops through the application of MAS and transgenic technologies would considerably enhance the economic viability of rural communities in which these crops are grown.

3.1.3.6 Estimated Cost of Research

We anticipate approximately $20 M will be required to realize the short, medium and long-term goals of this project.

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3.1.4 Evaluate & Utilize genetic variation to improve productivity

3.1.4.1 Background:

Genetic improvement of crop plants requires favorable recombination and selection of desired genes for multiple agronomically important traits. Genetic resources have been assembled for the legume crops (dry bean, pea, lentil and chickpea) worldwide and serve as a long term reserve for genetic variation. These collections have been mined for a number of desired genes, especially disease resistance and a number of traits conferring local adaptation to specific target environments. The focus on additional more specialized traits such as edible quality, nitrogen fixation efficiency and, more broadly, adaptation to changing climatic conditions affords additional opportunities to mine the available collections for favorable genetic variation that can be used to develop improved crop varieties.

3.1.4.2 Research Need/Problem Statement:

The pulse crops have been known to be a valuable source of nutrition for human and animal consumption for several millennia. In addition, they are known to provide significant contributions to cereal-based agriculture production systems. Despite these positive attributes, sufficient focus has not been placed on improvement of current pulse crop varieties for a number of quality attributes or nitrogen fixation. Available germplasm collections need to be evaluated for these traits and improvements made through development of improved varieties using conventional breeding methodology.

3.1.4.3 Approach Outline of Research Direction:

- Acquisition of seed from a broadly diverse collection of germplasm expected to contain the desired trait of interest.

- Identification and establishment of appropriate screening methodology and equipment to evaluate the germplasm accessions. (This will be quite varied depending on the specific trait being evaluated.)
- Evaluation and testing of the identified accessions in field and/or controlled conditions to establish proof-of-concept that the trait will be effective in accomplishing the desired outcome, i.e. increased nitrogen fixation, improved nutritional composition, greater adaptation to changing climatic conditions, etc.
- Hybridization and selection of improved breeding lines possessing the desired trait(s)
- Release of new varieties to producers and product testing by seed processors

3.1.4.4 Outcomes:

3.1.4.4.1 Immediate (2 years)

- Evaluation of germplasm collections for desired genetic variation.
- Identification of germplasm accessions possessing desired favorable genes for specific traits of interest.

3.1.4.4.2 Midterm (6 years)

- Incorporation of the favorable genes in breeding populations.
- Initial selection of breeding lines possessing the desired trait(s).

3.1.4.4.3 Long Term (10 years)

- Selection and release of new varieties possessing the desired traits.

3.1.4.5 Impacts:

3.1.4.5.1 Economical

- Release of improved varieties will reduce the need for costly inputs by producers.

3.1.4.5.2 Environmental

- Reduced potential for environmental contamination due to application of pesticides and fertilizers.

3.1.4.5.3 Sociological

- Improved consumer perception and acceptance of agriculture production and the food products they consume.
- Improved awareness of the conscientious public of the means and methods by which their food is produced and the care that is taken to protect the environment over the longer term.

3.1.4.6 Estimated Cost of Research

$5 M will be required to realize the short, medium and long-term goals of this project.

3.1.5 Pest Management in Pulse Crops

3.1.5.1 Background:

Crop diversification, reduced fallow periods, and limited inputs are being promoted in the Great Plains to improve economic and environmental sustainability in dryland cropping systems (Peterson et al., 1993). In Montana, USA, over 1.59 M ha or 36% of the dryland acreage for annual crop production was in summer fallow in 2003 (Montana Ag Statistics Service, 2004). Producers are encouraged to diversify crops away from monocultures, primarily wheat (Triticum *aestivum* L.), to reduce the area of land under fallow, and to reduce farm inputs, especially those that have negative impacts on economic and environmental sustainability (Matson et al., 1997; Struick and Bonciarelli, 1997; Gregory et al., 2002).

Water typically is the primary limiting factor for growing crops in wheat-based cropping systems in the semiarid northern Great Plains (NGP). Conventional summer fallow usually increases both soil water storage and nitratenitrate-N concentration for subsequent crop use. Summer fallow, however, is inefficient for precipitation storage, averaging only 25% efficiency in tilled systems (Farahani et al., 1998). Intensification of crop production by reducing summer fallow provides more efficient utilization of water in the semiarid central Great Plains (Farahani et al., 1998). Available N is the second most limiting factor for dryland crop production in semiarid agroecosystems (O'Leary and Conner, 1997). Soil nitrate-N availability is usually related to cereal yields. Increased nitrate-N content can also contaminate surface and groundwater due to N leaching and surface runoff. For decreasing fertilizer N applications and improving N utilization, producers are encouraged to diversify away from cereal monocultures, primarily spring wheat and durum (*Triticum turgidum* L. var. durum), to improve crop N uptake and reduce residual soil N and N leaching. Additionally, purchasing fertilizer N is a significant expense for producers. Improved nutrient-use efficiency, particularly N, is an important goal in cropping systems (Karlen et al., 1994; Raun and Johnson, 1999). Huggins and Pan (2003) showed determination of key indicators of N use efficiency (NUE) in cereal-based agroecosystems enabled broad assessment of agronomic management and environmental factors related to N use. Key indicators of NUE include N in grain and N aboveground biomass, N harvest index, and grain N accumulation efficiency.

Pulse crops are well adapted to semiarid NGP environments (Hedel and Helm, 1993; Carr et al., 1998, 2004; Lenssen et al., 2007). Replacing summer fallow with pea and lentil may be an effective cropping system to improve soil quality and producers' returns. Pulse crops rarely show a yield response to applied N and consequently almost never receive N fertilizers. Due to their short growing seasons, pea and lentil typically utilize less soil water than do grain and oilseed crops (citations). The successful inclusion of pulse crops preceding cereals is now well documented in several dryland environments. Thomson et al. (1997) showed that white lupine (*Lupinus albus*), blue lupine (*L. angustifolius),* faba bean (*Vicia faba*), and field pea (*Pisum sativum*) produced satisfactory seed yields following wheat in Western Australia. In Saskatchewan, Canada, Miller et al. (2002a, 2003a, 2003b) documented that field pea, lentil (*Lens culinaris*), and chickpea (*Cicer arietanum*) were excellent crops in sequences with spring wheat. Additionally, Miller et al. (2002b) and Gan et al. (2003) documented increased grain yield and protein of spring wheat following these pulses compared to spring wheat following spring wheat. Unkovich and Pate (2000) reviewed recent literature on symbiotic nitrogen fixation by annual legumes, and concluded that pea generally fixed more N than lentil or chickpea. In a review of Australian research, Evans et al. (2001) concluded that blue lupine and field pea typically had positive effects on nitrogen balances.

Pulse producers and researchers in Montana and North Dakota have observed that pulses often are weedier crops than wheat. Part of the reason for that perception is that wheat crops are almost never land rolled because cutting height at harvest typically is above exposed rocks. Land rolling increases early season emergence of green foxtail and a suite of broadleaf weeds, including prickly lettuce (*Lactuca serriola*), horseweed (*Conyza canadensis*), kochia (*Bassia scoparia*), Russian thistle (*Salsola iberica*), and the crucifers flixweed (*Descurainia sophia*) and tumble mustard *Sisymbrium altissimum*) that often are not well controlled by preplant herbicide applications in field pea, lentil, or chickpea (Lenssen, 2008). Lenssen (unpublished results) found that Russian thistle biomass explained 43% of variation in pea yield. . Because of their poor competitiveness with weeds, pulse production is recommended for sites that have lower weed pressure. However, few fields are weed-free and few post-emergence broad leaf herbicides are registered for pea, lentil or chickpea. Lentil producers rely solely on preplant applications of glyphosate, ALS inhibitors and dinitroaniline herbicides. A survey of dryland farms in Montana suggested that over 70% of nearly 700 farms had ALS inhibitor-resistant kochia present. The recent finding and likely spread of glyphosate resistant kochia in Kansas (Heap, 2010) may greatly impact zero tillage production of pulse and other crops in semiarid regions.

Diseases and insect pests also significantly limit pulse production. Important diseases include foliar diseases (Ascochyta blights, Botrytis gray mold, anthracnose and Sclerotinia stem rot), root diseases (Fusarium wilts, Aphanomyces root rot, Rhizoctonia and Fusarium root rots, and root diseases caused by nematodes) and virus diseases (Pea Enation mosaic virus, bean leaf roll virus, and pea streak virus). Insect pests include pea leaf and pea weevils, aphids, wireworms, loopers, cutworm and armyworms.

3.1.5.2 Research Need/Problem Statement:

Weeds, insects, and diseases negatively impact pulse productivity, quality, end-market use, and producer net income. Herbicides are the single highest direct cost for pea and lentil production in the northern Great Plains as well as other areas. Integrated strategies to concomitantly improve crop competitiveness against weeds and decrease herbicide inputs would improve economic and environmental sustainability of NGP producers and communities. Multi-tactic cultural practices can greatly reduce the requirement for herbicide applications (Anderson, 2005). The widespread occurrence of herbicide resistance to commonly occurring, competitive weeds evinces the need to develop effective weed management strategies with reduced reliance on herbicides.

Post-emergence broadleaf weed control in lentil is unavailable. Broadleaf weed management is accomplished solely by pre-plant applications of an ALS inhibitor, Pursuit®, sonalan, and glyphosate. Prickly lettuce and kochia frequently infest lentil fields and require preharvest applications of glyphosate or paraquat, increasing selection pressure for herbicide resistance. Improved cultural and rotational strategies to decrease selection pressure for herbicide resistance are necessary to improve long-term sustainability of pulse production in the NGP.

Crop sequencing can be an effective tool in reducing weed interference (Anderson et al., 1999). Some sequencing strategies have been well researched and documented to improve weed management, but none have specifically targeted improving pulse production systems. For instance, the influence of stacking pulse crops on weed and disease management has not been researched in the NGP. Additionally, few long-term cropping systems studies have been conducted investigating economic and ecological sustainability of diversified pulse systems in regards to pulse production systems.

3.1.5.3 Approach Outline of Research Direction:

Solutions for improved weed, disease, and pest management will be developed by research-based investigations with different pulse crops. Short-term, replicated studies will test individual and multi-tactic cultural weed management strategies with known seed bank densities of specific weeds of interest. Long-term replicated studies will compare pulse production in different rotation configurations, including stacked- and alternate-year rotations, with different numbers of growing seasons between pulse crops. Specific research objectives will include:

1) Development of dryland and irrigated weed and disease management strategies for various pulses to minimize yield impacts and risk to growers.
2) Development of multiyear dryland and irrigated cropping strategies that include specific rotations and sequences of various pulse crops that utilize cultural practices and decrease selection pressure for herbicide/fungicide resistance in common weeds and disease causing organisms to economically and environmentally optimize productivity.
3) Screening commercially available chemicals for efficiently managing foliar diseases and insects.
4) Utilization of mutagenesis and/or genetic modification for developing herbicide-resistant pulse crops.
5) Discovery and exploitation of genetic sources of resistance to diseases and insects, and incorporation of the resistance sources into elite cultivars.

3.1.5.4 Outcomes:

3.1.5.4.1 Immediate (2 years)

- Determine potential pulse production in relation to competition by various weeds.
- Development of management principles that are common to agricultural systems that reduce risk, and improve economic and environmental sustainability.
- Identification and selection of resistance sources in cultivated and wild relatives of pulse crops for important diseases for incorporating resistance genes into elite cultivars.
- Identification and recommendation of fungicides with different modes of action for managing foliar diseases.
- Initiation of population studies of important fungal pathogens from different production regions of the United States.

3.1.5.4.2 Midterm (6 years)

- Initiate technology transfer programs to provide educational materials through appropriate venues on the effectiveness and economic and environmental benefits of cultural weed (pest) management strategies.
- Identification of new potential strategies to reduce production costs and risks of economic loss.

- Increased contribution of pulse crops to dryland farm income in semiarid regions of the USA.
- Development of forecasting models of virus-transmitting insects.
- Development and release of pulse cultivars with resistance to some of the important diseases.
- Development and testing mutants and/or GM pulse crops for herbicide resistance.
- Detailed knowledge of pathogen biology and genetic variation among different production regions.
- Increased acreage sown to pulse crops in US, resulting in an increase in exports.
- Management strategies based on knowledge of weed seed bank, plant diseases, arthropod pests, and water availability affect yield, quality, and profitability of dryland cereal-pulse crop production systems with reduced purchased fertilizer and other agricultural chemical inputs.
- Mechanistic crop simulation tools and databases to evaluate the immediate and long-term effects of various weed management systems.

3.1.5.4.3 Long Term (10 years)

- Evaluate long-term sustainability of alternative food production systems to climate and energy cost variability, transportation costs, etc.
- Development of economic risk averting management strategies that improve soil productivity and quality, enhance soil and water conservation and nutrient cycling, and reduce fuel and pesticide use while enhancing the natural resource base.
- Identification of reduced risk strategies for diversified irrigated and dryland cropping sequences that includes pulses and bioenergy crops.
- Identification of optimal rotation sequences that reduce reliance on off-farm inputs to increase the profitability of production systems.
- Comprehensive management plans for important diseases and pests of pulse crops.
- Established recommendations of practices for managing important weeds of pulse crops.

3.1.5.5 Impacts:

3.1.5.5.1 Economical

We estimate that at least 10% of the annual yield potential of pulse crops grown in the US is lost due to diseases, weeds and pests. Based on crop values for 2009, this would be a loss of approximately $110 M. A considerable portion of these potential losses would be eliminated through the development of new agronomic management strategies for controlling losses due to these adverse production factors.

3.1.5.5.2 Environmental

We anticipate that the development of new agronomic management strategies for controlling losses due to diseases, pests and weeds would result in an increase in the acreage of pulse crops produced in the US. This would result in a reduction in fertilizer, pesticide and herbicide usage in these areas of cultivation, as pulse crops typically require very little inputs of these agrochemicals as compared to other crops. Methods for controlling virus diseases of pea would reduce the amount of insecticide applied annually for controlling vectors of these viruses. New methods for controlling losses in chickpea due to Ascochyta blight and losses in common bean due to Sclerotinia white mold disease would nearly eliminate the use of fungicides for these crops. This would result in improved soil and water quality and would also reduce health risks to non-target organisms, including humans, associated with exposure to these agrochemicals.

3.1.5.5.3 Sociological

The ability to expand the range of areas in the US under production of pulse crops through the application of MAS and transgenic technologies would considerably enhance the economic viability of rural communities in which these crops are grown.

3.1.5.6 Estimated Cost of Research:

$10 M will be required to realize the short, medium and long-term goals of this project.

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3.1.6 Develop systems that utilize organic methods and pulses.

3.1.6.1 Background:

Pulses can both supply the majority of human dietary protein, and provide the nitrogen needed by sustainable agricultural systems. By disallowing the use of synthetic nitrogen fertilizers, organic agriculture is highly dependent on legumes for nitrogen supply. These systems can be optimized to serve as model agricultural systems independent of synthetic nitrogen inputs. Particularly in developing regions, organic agriculture can and should be a significant portion of the agricultural development (UNEP-UNCTAD, 2008). The development of highly productive organic systems is needed to provide sustainable food and fertility globally.

3.1.6.2 Research Need/ Problem Statement:

Research and develop a global array of highly productive organic agricultural systems that use pulse crops as the primary nitrogen source.

3.1.6.3 Approach / Outline of Research Direction:

Each major current and potential agricultural eco-region would develop one or more model systems. Local producers would be engaged with researchers to redesign and optimize new systems that produce maximum human food value with high energy and input efficiency, utilizing pulse crops as both a food and fertility source.

3.1.6.4 Outcomes:

3.1.6.4.1 Immediate (2 years)

- Identification of model trial sites, partners, system concepts and outlines. Field application and related research initiated.

3.1.6.4.2 Midterm (6 years)

- Preliminary reports and analysis of production, energy and input efficiency, economic viability.
- Minimum of 10 commercial units near each trial adopt one of the models, leading to a minimum 20% increase in food output: energy input ratio.

3.1.6.4.3 Long Term (10 years)

- Adoption of high efficiency systems by at least 5% of agricultural production areas, providing sustainable food self-sufficiency in current food-deficit areas and energy savings in export regions.

3.1.6.5 Impacts:

3.1.6.5.1 Economical

Sustainable organic farm models must be economically viable in order to be adopted. Costs of production per unit food value would decline.

3.1.6.5.2 Environmental

Land areas adopting these models would dramatically reduce use of fertilizers, pesticides, and herbicides. These changes if adopted on sufficient land base would stabilize global pollinator insect and amphibian populations, and over time reduce waterway hypoxia and climate change.

3.1.6.5.3 Sociological

Improved food self-sufficiency in multiple ecoregions will strengthen global security.

3.1.6.6 Estimated Cost of Research:

We anticipate approximately $10 M annually will be required to realize the short, medium and long-term goals of this project.

3.1.6.7 Bibliography

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3.2 Utilizing Pulse Cropping Systems to Adapt to Global Climate Change

3.2.1 Increase roles of pulses to reduce greenhouse gases (GHG)

3.2.1.1 Background

Carbon is a key currency for modern agriculture linked to climate-altering greenhouse gases. Cropping system carbon is accounted for in two major ways; by measuring change in soil carbon stocks (i.e. increase carbon 'sequestration') and by examining energy outputs relative to inputs. Agriculture is estimated to account for about 90% of anthropogenic nitrous oxide emissions, and 25% of carbon dioxide emissions (Duxbury 1994). Nitrogen fertilizer is the main source of nitrous oxide emissions in dryland grain rotations. Since pulse crops do not need commercial nitrogen fertilizer, they have the potential to significantly lower overall emissions of this potent greenhouse gas (GHG). (Nitrous oxide is approximately 300 times more potent than carbon dioxide as a GHG.)

In a recent study that measured both nitrous oxide and carbon dioxide emissions for a common dryland grain rotation, GHG in the form of nitrous oxide emissions from fertilizer applications often outweighed any carbon sequestration from crop growth (Stöckle et al., 2010). Even under reduced tillage scenarios, such as replacing traditional summer fallow production involving multiple tillage passes with chemical fallow (several herbicide applications), the net effect of carbon sequestration and nitrous oxide emissions often resulted in a net source, not a sink, of GHG emissions.

Stockle et al (2010) compared two typical annual dryland cropping rotations for the eastern Washington growing region. GHG emissions were evaluated under various reduced tillage scenarios for a traditional winter wheat, spring grain, spring pea rotation and a more intensive spring wheat, winter wheat, spring barley rotation In the pulse rotations, simulated N_20 emissions were lower than in the intensive grain rotation, and this result was correlated with the lower quantity of fertilizer applied. Because this GHG is so much more potent than carbon dioxide, fertilizer application can easily overwhelm carbon benefits achieved through reduced tillage or grain-intensive rotations. It appears that GHG emissions would be minimized by producing the traditional rotation using no-tillage practices than in the more intensive system. However, because pulse crops leave little crop residue, rotations with pulses have less carbon sequestration than rotations with only small grains. It should be noted that a continuous cropping system without fallow or a cropping system that contains cover crops, perennials, or green manure crops which would fit into a pulse crop production system may increase carbon sequestration despite less crop residue, primarily due to increased rhizosphere activity (Khan et al., 2007)

An economic evaluation of these two rotations concluded that the traditional rotation was more profitable than the rotation with pulses, using 5-year average crop prices (Painter, 2010). Growers try to maximize the proportion of grain in their rotations, as these crops are typically more profitable than the pulse crops. However, in recent years pulse prices have become more competitive, particularly for garbanzos. If growers are charged a carbon tax on their nitrogen fertilizer, it will increase the relative profitability of pulse crops.

3.2.1.2 Research Need/Problem Statement.

Possible methods for increasing cropping intensity in an economically sensible manner include replacement of summer fallow with pulse crops and growing pulses during spring and fall shoulder season periods where the length of season permits. Indeed pulses have been responsible for replacing significant summer fallow area in the northern Great Plains over the last 10 -15 yr (Tanaka et al, 2010). To meet this C sequestration goal, further research needs to be conducted to improve the adaptation of pulse crops to the driest regions so that remaining vestiges of summer fallow can be economically transformed. Also, adaptation of pulses to fill shoulder season niches needs to be aggressively explored to find economically optimal strategies for capturing this additional C sequestration opportunity.

The role of annual pulse crops has not been well studied with respect to soil carbon; long-term cropping systems research that includes pulse crops is rare. Main drivers of soil carbon sequestration are no-tillage management and increased cropping intensity (increased biomass C inputs to system per multi-year period). Pulses may play a pivotal role in carbon accretion in semiarid North American wheat-based systems by enhancing the economic viability of no-till systems, and by increasing crop intensity by replacing summer fallow area or by contributing fall or spring 'shoulder season' biomass. However, some scientists have argued that the relatively narrow C:N ratio of pulse crops stimulates decomposition of soil organic carbon. Thus, despite some long-term records that show soil carbon gain under cropping systems that include pulse crops (Zentner et al., 2001; Campbell et al., 2007; Sainju et al., 2009), the rate of carbon gain (or loss) remains uncertain, as does the influence of the soil-climate context.

Research on this topic is in the developmental stage at this point, but would greatly benefit from a regional coordinated approach. Many decisions need to be made regarding soil depth and timeframes for measurement. Field measurements and laboratory analyses are very time-consuming and expensive. Additional research is needed in order to calibrate modeling of carbon and nitrogen cycling in cropping systems across many pulse growing regions.

In addition to calibration of GHG measurements, further research is needed to identify how different pulse rotations can contribute to lowering GHG emissions in agriculture under different policy scenarios. For example, how should carbon credits or other policy instruments be structured to induce the production of crops that reduce GHG emissions? Perhaps a tax on nitrogen fertilizer is the most cost-effective method for reducing GHG emissions, but taxes are politically unpalatable. Carbon credits would have to be very high to induce changes in behavior in areas with little capacity for sequestration, including large areas of arid and semi-arid dryland wheat production in the West.

3.2.1.3 Approach Outline of Research Direction

Immediate and midterm outcomes for this aspect of the problem must include modeling of carbon and nitrogen processes for pulse cropping systems. Field measurements and computer modeling along the lines of the CropSyst program (Stöckle et al., 1994; Stöckle et al., 2003) are needed in order to document the environmental changes of increasing the proportion of pulses in our cropping systems. Once likely environmental changes have been established, economic impacts of these benefits can be calculated. Costs and benefits to society of current and proposed cropping systems can then be compared. Policy strategies to achieve these benefits can then be developed that will help reduce greenhouse gas production from agricultural systems at least cost to society while maintaining a profitable agricultural sector.

3.2.1.4 Outcomes:

3.2.1.4.1 Immediate (2 years)

- Identify pulse crop varieties that can be grown during 'shoulder' seasons.

3.2.1.4.2 Midterm (6 years)

- Enhance systems models and decision aids that evaluate the potential of pulse crop productions for global food security while maintaining soil and environmental quality.
- Develop sustainable crop production through integrated pest management and reduced use of purchased nitrogen fertilizers in cropping systems.
- Create decision support tools to assess the collective impacts of pulse production on soil nutrient levels.

3.2.1.4.3 Long Term (10 years)

- Model C and N movements in pulse systems for different regions under various tillage scenarios that includes the costs and benefits of these systems based on economic and environmental data and the affects of policies such as carbon credits, taxation, green payments, etc. in terms of economic and environmental outcomes.

3.2.1.5 Impacts:

3.2.1.5.1 Economical

- Reduce production costs through reduced synthetic fertilizers and pesticides; provide another crop for consumer healthfulness and enhanced revenue

3.2.1.5.2 Environmental

- Reduce greenhouse gas emissions associate with use of chemically synthesized nitrogen fertilizers.

3.2.1.5.3 Sociological

- Improved societal perspective of the agricultural production systems.

3.2.1.6 Estimated Cost of Research

We anticipate approximately $ 4 M annually will be required to realize the short, medium and long-term goals of this project.

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3.2.2 Optimize carbon and energy budgets.

3.2.2.1 Background

Energy price volatility threatens economic stability of U.S. agriculture; chiefly N fertilizer (CAST 2010). For example, N fertilizer alone accounted for 47% of the crop energy inputs for all U.S. wheat production (Piringer and Steinberg 2006); and the proportion accounted by N fertilizer is considerably greater for high protein wheat production common to the northern Great Plains where pulse crops are increasing in importance. Where utilized, pulses promise to reduce regional cropping system energy budgets by 15-20% through biological N fixation. Little or no N fertilizer is used during the pulse crop production year, and N use efficiency for subsequent non-legume crops is typically increased. Further energetic gains may be realized where pulses facilitate reduced and no-tillage systems; and reduce cereal crop pests.

U.S. agriculture has been challenged to maintain or increase its productivity while shrinking its environmental footprint; clear goals have been set to increase C sequestration by 15% while reducing energy, nitrogen, and water use by 10% (USDA 2010). N-fixing pulse crops, when used in cereal-based cropping system, hold genuine promise for meeting or exceeding these goals. It has been shown in a few long-term studies that biomass C input from pulse crops is as valuable to soil organic C accretion as that from cereal crops (Zentner et al., 2001; Campbell et al., 2007; Sainju et al., 2009). Thus, if pulses can be used in ways that sensibly increase the overall biomass C input within a cereal-based cropping system, increased C sequestration within the soil is expected to be roughly proportional to the rate of increase in biomass C input (Campbell et al., 2007).

A 2009 review of 12 successful pulse growers in northeastern Montana found that wheat yield following pulse crops was increased by an average of 40%, while reducing N fertilizer application by an average of 15%, compared with wheat grown on cereal stubble (Mac Burgess, unpublished data). The energy budget implications for this comparison were very significant, by increasing grain outputs with reduced inputs. This farm field-scale study followed on plot-scale research conducted at Bozeman, MT, that showed annual energy inputs were decreased 15–20% for pulse – wheat systems during 2005–2006 compared with wheat-only systems, while net energy gain was maintained equal (unpublished data). Given that nitrogen is the dominant energy input into U.S. cereal production systems, every effort should be made to learn how biologically fixed N can offset petroleum-derived fertilizer N in economically sensible ways. Pulse crop impacts on cereal cropping system energy budgets should be quantified from semiarid to subhumid regions so that this crucial energy angle is fully captured.

3.2.2.2 Research Need/Problem Statement

Soil N contribution from pulses remains less well understood than is necessary to provide land managers with reliable spatial and temporal predictions for cereal crop growth (Walley et al. 2007). In a 2009 pulse crop and wheat grower survey conducted in NE Montana, where pulse crop production is a recent phenomenon, we were surprised to learn of the tremendous variation among growers in estimating pulse-derived soil N credits for a subsequent wheat crop. Some ascribed a ‘zero’ N benefit, thinking the major rotational benefit was from disrupting wheat pests, and were content to capture a N response through increased wheat protein which provides valuable price premiums in many years. Others ascribed a N benefit that was substantially larger than that observed in replicated small plot research. So the average reduction of 15% in fertilizer N appears to be a highly misleading 'average'. Another grower in Montana, a leading mind for variable rate N application in wheat production, has been unable to implement variable rate N application on pea stubble, due to our poor understanding of the spatially variable nature of legume growth, N fixation and soil N contribution. Thus, in depth research is needed to understand spatial and temporal aspects of soil N contribution from pulse crops, to full capture this benefit.

In semi-arid rainfed systems grain yield, water-productivity (also called water use-efficiency (WUE)) is driven strongly by the numerator, since all crop available soil water is used each year in annually cropped systems (Miller et al. 2002.) Improved knowledge of the potential for pulses to reduce energy constraints to agriculture carries important farm policy implications critical to incentivizing sustainable energy use.

3.2.2.3 Approach Outline of Research Direction

The role of pulses in cropping system energy budgets will be identified in replicated long-term plot-scale systems studies and by auditing (voluntary) established farming systems at the field scale. Data combining these temporal and spatial aspects will be combined to model aggregate impacts on energy use associated with different pulse-based cropping systems in North America. Cropping system studies include pulses used for cover crops, and forage or grain harvest to quantify reduction in crop energy inputs, and changes in energy use efficiency and a real net energy gain. Understanding net change in soil carbon and nitrogen pools is a necessary and key aspect to cropping system energy accounting.

3.2.2.4 Outcomes:

3.2.2.4.1 Immediate (2 years)

- Survey current and potential pulse production areas for conditions which currently limit pulse productivity.

3.2.2.4.2 Midterm (6 years)

- Determine regionally-specific management strategies to enhance the production and quality of pulse crops
- Identify mechanistic crop and soil simulation tools and databases to evaluate the economic effects, resource use, and environmental impacts of strategies for irrigated and dryland pulse crop production.
- Enhance systems models and decision aids that evaluate the potential of pulse crop productions for global food security while maintaining soil and environmental quality.
- Create decision support tools to assess the collective impacts of pulse production on soil nutrient levels.

3.2.2.4.3 Long Term (10 years)

- Model C and N movements in pulse systems for different regions under various tillage scenarios that includes the costs and benefits of these systems based on economic and environmental data and the affects of policies such as carbon credits, taxation, green payments, etc. in terms of economic and environmental outcomes.

- Model aggregate cropping system energy budgets at regional, national, and continental scales.

3.2.2.5 Impacts:

3.2.2.5.1 Economical

Reduce production costs through reduced synthetic fertilizers and pesticides; provide another crop for consumer healthfulness and enhanced revenue

3.2.2.5.2 Environmental

Reduce need for commercial nitrogen fertilizers.

3.2.2.5.3 Sociological

Improved societal perspective of the agricultural production systems.

3.2.2.6 Estimated Cost of Research

$ 5 M annually will be required to realize the short, medium and long-term goals of this project.

3.2.2.7 Bibliography

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3.2.3 Efficient use of available water

3.2.3.1 Background

As the thirsty competition swells in the form of increasing population, urbanization, environmental consciousness, recreation and tourism, and related concerns, agriculture's access to a critical resource is no longer guaranteed. Water scarcity is especially evident in the prime agricultural areas of the arid and semi-arid areas, but it is also being felt in the humid regions of the world. Solving these issues will require the continued refining of water conservation measures including improved efficiency of irrigation water application systems and improved water productivity of all crops. For instance, growing pulse crops in a water limited future will require substantial investments in research to evaluate water productivity under deficit water conditions for drought tolerant pulse varieties and as part of various cropping systems.

Production of grain legumes such as dry peas, lentils, and chickpeas are typically grown under dryland conditions in arid and semi-arid regions. Dry bean production in the more arid regions (e.g., CA, WA, OR, MT and ID) is typically irrigated; however, substantial rainfed dry production also occurs in more humid regions (e.g., WI, MN, MI and ND). These growing regions differ tremendously in the rainfall amounts and patterns, but the best management of the available water is critical to all.

Cultural practices such as conservation tillage, adjusting planting dates and densities, and improved drought tolerant varieties and better pest control will affect crop productivity and water use for both irrigated and dryland crops, but much of this information is lacking for cropping systems that include pulses. In some areas, cropping strategies such as double-cropping, inter-cropping, relay cropping, crop specific rotations and sequences can take advantage of the lower water demand of certain crops, particularly some pulses, and times or periods with higher rainfall to increase total productivity. Pulses can also be used as cover crops to supplement soil nitrogen needs and reduce fertilizer costs. Improving soil nutrient status, in particular soil nitrogen availability, has been implicated in increases in water use efficiency by reducing water loss from roots in the process of obtaining soil nutrients. More studies are needed to further understand and identify the mechanisms involved in this process.

3.2.3.2 Research Need/Problem Statement.

Management of soil water is a major constraint when introducing new crops into existing systems. Continuous, every year dryland cropping that replaced a small grain-fallow system would likely reduce potential yields for both the grain and the pulse crops because of reduced total soil water availability over the 2 year period. Every acre of fallow replaced with a relatively high water use crop such as chickpea or lentils will probably decrease subsequent small grain yields by 30%-40% below historical averages under arid and semi-arid conditions. New knowledge is needed to quantify the yield and ecosystem effects over a range of more intensive farming practices and strategies including conservation tillage, adjusted planting dates and densities, and improved varieties and better pest control alternatives.

Demands to conserve water by domestic, industrial, environmental and agricultural users are escalating, and many pulse crops including dry beans are irrigated. Carefully managed deficit irrigation on agronomic crops including pulses provides the greatest potential for substantially reducing agricultural water use, but this knowledge is lacking for pulses in most areas of the world. Thus, it is imperative to develop higher levels of irrigation management and systems that utilize soil water more efficiently can potentially reduce operating costs as well as energy and water use.

3.2.3.3 Approach Outline of Research Direction

Investigations establishing crop water use and water productivity of different pulse crops and varieties are necessary for meeting the goals of this endeavor.

1) Development of dryland and irrigated water management strategies for various pulses in water limited cropping systems to minimize adverse yield impacts and risk to growers.
2) Determination of the actual water use requirements of many annual pulse crops, which are not well defined, and are needed for improved cropping systems management as well making potential production assessments for different regions in different years.
3) Development of multiyear cropping strategies that include crop specific rotations and sequences including various pulse crops that can take advantage of cultural practices and maximize precipitation use efficiencies to economically and environmentally optimize productivity.

3.2.3.4 Outcomes:

3.2.3.4.1 Immediate (2 years)

- Develop county level land availability, crop requirements, soil suitability, and climate and water resources for cool season pulse crops.
- Establish site-specific sprinkler systems to impose a range of abiotic stresses at each location as an efficient screening method to identify genotypes tolerant of stresses such as drought and heat in multiple regions.

3.2.3.4.2 Midterm (6 years)

- Increase contribution of pulse crops to dryland farm income in semi-arid regions of the USA.
- Develop tillage guidelines that assess the sustainability of reduced tillage in irrigated and rain-fed pulse production systems.
- Identify specific soil and residue management practice recommendations for dryland pulse production in the semi-arid regions to improve soil water retention and productivity.
- Develop new decision making technology for selecting sustainable systems that include alternative and specialty crops under limited water conditions.

3.2.3.4.3 Long Term (10 years)

- Develop economic risk averting management strategies that improve soil productivity, enhance soil and water conservation and nutrient cycling, and reduce fuel and pesticide use while enhancing the natural resource base.
- Identify reduced risk strategies for diversified irrigated and dryland cropping sequences that include pulses and bioenergy crops.

- Identify whole-system management strategies based on enhanced agroecological functions when including alternative crops under water limited rotation systems to reduce income variability and risk of economic loss to producers

3.2.3.5 Impacts:

3.2.3.5.1 Economical

Increase production capacity by allowing pulses to be grown in more dryland environments. This will increase the sustainability of rural communities

3.2.3.5.2 Environmental

Reduced soil loss, fossil fuel use, pesticide use, and greenhouse gas emission while improving the sustainability of the cropping system through better water and nutrient use.

3.2.3.5.3 Sociological

The ability to expand the range of areas in the US under production of pulse crops through more efficient use of available water would considerably enhance the economic viability of rural communities in which these crops are grown.

3.2.3.6 Estimated Cost of Research

Approximately $ 4 M annually will be required to realize the short, medium and long-term goals of this project.

3.2.4 Broaden agro-ecological adaptations

3.2.4.1 Background

Viable dryland cropping systems in the future that include pulses such as peas and lentils will depend on the adaptability of all crops in these systems to the changing growing conditions and stresses due to climate change in each region. However, pulse crop production areas are limited due to various environmental pressures. Preliminary data on edible types of winter peas and lentils indicates high potential for their use in the cropping systems. The necessary genes for winter hardiness available in pea germplasm have been used to convert the Austrian winter pea to a white-flowered edible type of winter pea. Similarly, germplasm of winter hardy lentil is available for development of market quality edible types that can be fall sown. Development of the winter legume varieties has been accomplished with emphasis on traits necessary for food and feed. Yield of these newly developed winter hardy types have been very promising and offer the industry a new approach to improving yield and profitability. Yield increases over traditional spring sown types have ranged from 40 to over 100% depending on the year and survival.

Winter legumes would, by necessity, have to be planted in a minimum tillage or direct seeding system for the purpose of erosion control. The use of winter legumes in direct-seeded cropping systems is beneficial to yield and also provides an excellent break crop for cereal disease control. Other benefits from the use of winter legumes include better weed control and improved soil nutritional status. Also, growers have indicated they would prefer to seed the legume crops in the fall to avoid problems associated with spring seedbed preparation such as cold and generally wet soil conditions. Sowing legume crops in the fall would be a definite advantage for growers.

Based on preliminary evaluations, winter hardy peas and lentils are well adapted to direct seed cropping systems and it appears that the additional residue from the cereal crop provides added protection from cold winter temperatures. Additional benefits from fall seeding include earlier crop growth during a cooler part of the season and improved water use efficiency. Fall sown peas and lentils flower in a cooler part of the season and seed set has generally been excellent. Because of earlier crop development, winter peas and lentils have matured about 1 to 2 weeks earlier than comparable spring sown crops.

To make edible winter peas and lentils a viable crop for the cereal-based cropping systems across the northern tier US states additional breeding and agronomic studies need to be performed with specific attention on adaptation, disease resistance, seed quality factors and yield. The prospect of winter peas with edible quality traits has been studied over the past decade with some promising results. The semi-leafless trait has been incorporated into semi-dwarf winter peas with white flowers and clear seed coats. Additional work needs to be done on these types to improve seed appearance, edible quality, cooking quality traits, disease resistance and yield.

3.2.4.2 Research Need/ Problem Statement.

A high priority need is to increase the potential land base and production capacity for pulse crops by development of pulses and nitrogen-fixing symbionts that are productive in agro-ecological conditions currently considered marginal or unsuited to their production. Low soil pH reduces viability and activity of the rhizobial bacterial partners required to supply pulse nitrogen. Low precipitation reduces grain productivity and viability. Excess heat during the growing season can reduce photosynthesis and growth. Cold soil temperature at planting can favor seedling diseases and reduce stands. In order to increase the available land area where pulses can be profitably and productively grown, crop varieties and symbiont strains must be selected and adapted to withstand and thrive in adverse conditions. Specific needs are:

1) Expansion of production into low rainfall climates which will require new mechanisms to take advantage of the limited precipitation.
2) Heat stress tolerance in low rainfall environments to increase adoption and expansion of legumes.
3) Weed control methods for cropping systems with pulses
4) Adaptation and methods for stand establishment of winter legumes in no-till cropping systems.

3.2.4.3 Approach Outline of Research Direction

1) Evaluate germplasm available worldwide for adaptation to US production environments.
2) Establish field-based and controlled environment selection methods for improved winter hardiness.
3) Experiments focused on rotational and cropping system, especially no-till, benefits of the winter pulse crops in production systems.

3.2.4.4 Outcomes:

3.2.4.4.1 Immediate (2 years)

- Expand global collections of pulse crops into marginal environments.
- Establish a systems approach to identification of improved winter legume cultivars.
- Predict potential production for regionally grown pulse crops.

3.2.4.4.2 Midterm (6 years)

- Increase contribution of pulse crops to dryland farm income in semi-arid regions of the USA.
- Increase acreage sown to pulse crops in US, resulting in and an increase in exports
- Assess global core genetic collections for tolerance to limiting conditions and initiate selective breeding.
- Increase adoption of no-till across the pulse growing regions reducing production costs for producers

3.2.4.4.3 Long Term (10 years)

- Increase profitable pulse production areas by >10 million acres and potential pulse productivity on >100 M acres of marginal lands by >20%.

3.2.4.5 Impacts

3.2.4.5.1 Economical

Increased production capacity valued at a minimum of $1 B annually

3.2.4.5.2 Environmental

We anticipate that increasing the range of adaptation of pulse crops would result in an increase in the acres planted in these crops in the US. This would result in a reduction in fertilizer, pesticide and herbicide usage in these areas of cultivation, as pulse crops typically require very little inputs of these agrochemicals as compared to other crops.

3.2.4.5.3 Sociological

The ability to expand the range of areas in the US under production of pulse crops through the US would considerably enhance the economic viability of rural communities in which these crops are grown.

3.2.4.6 Estimated Cost of Research

$ 12 M annually will be required to realize the short, medium and long-term goals of this project.

3.2.5 Cropping systems to enhance soil resources

3.2.5.1 Background

By approaching crop production systems as integrated systems where above- and belowground processes are linked, management techniques for enhance production and global food security under a changing climate will emerge (Hanson et al., 2007). Pulse crops provide mechanisms to improve nutrient, particularly nitrogen, status of the soil, utilize innovative crop production techniques such as no-till planting and poly- or inter-cropping, and create a window for inclusion of cover crops, green manures, and forage crops into systems, even under short growing seasons. Previous research in the northern Great Plains has shown the positive effects of dry pea and lentil in the rotation, particularly related to precipitation use efficiency (Krupinsky et al., 2006; Tanaka et al., 2007). However, the impact of pulses on weed and disease cycles has been under examined (Krupinsky et al., 2007). Studies conducted thus far typically have a very narrow eco-range and rarely compare similar characteristics. Therefore, to identify a cropping system which is economically and environmentally sustainable and has a global impact, an experiment must be designed to measure a set of standard parameters while implementing regionally-specific production protocols.

3.2.5.2 Research Need/Problem Statement.

Specific research needs in this area include:

1) Identifying the appropriate, regionally-specific cropping systems – pulse crop, crop variety, rotation, etc. – for inclusion of pulse crops to:
   a. Optimize water-productivity and nutrient-, and energy-use efficiencies,
   b. Enhance production and nutrient status of pulse crop as well as other crops in the rotation,

c. Develop innovative productions practices, such as utilizing no-till techniques or providing a window for cover crop implementation, and
d. Reduce fossil fuel use and greenhouse gas emission.

3.2.5.3 Approach Outline of Research Direction

Experiments highlighting two or more pulse crops and comparing current, best management practices to one or more innovative, alternative management practices will be implemented for a minimum of five years in differing eco-regions throughout the U.S. Parameters such as crop yield and protein content, precipitation use efficiency, soil carbon, total and mineralizable nitrogen, and energy use for production will be measured at each location. In addition, pest concerns and severity will be noted. Other soil parameters such as soil moisture, aggregation, rhizosphere activity and diversity, and greenhouse gas emissions may be determined. Also, fiber content as well as minerals, such as iron, magnesium, and zinc, and antioxidants may be measured in grain legumes.

3.2.5.4 Outcomes:

3.2.5.4.1 Immediate (2 years)

- Predict potential production for regionally grown pulse crops
- Develop county level land availability, crop requirements, soil suitability, and climate and water resources for cool season pulse crops.
- Develop a set of management principles that are common to agricultural systems across production regions that reduce risks, improve competitiveness, and promote environmental stewardship.
- Initiate technology transfer programs to provide educational materials through appropriate venues on the benefits of pulses in American diets.
- Identify potential strategies and technologies to reduce production costs and risks of economic loss.
- Identify regionally-specific resource concerns – soil health, pests, or water quality – for breeding and genetic modifications to address

3.2.5.4.2 Midterm (6 years)

- Develop sustainable crop production through integrated pest management and reduced use of purchased nitrogen fertilizers in cropping systems.
- Identify management strategies based on knowledge of how soil properties, plant diseases, and water availability affect the yield, quality, and profitability of dryland small grain-pulse crop production systems that reduce commercial fertilizer use, and minimize the use and negative impacts of agricultural chemicals.
- Identify establishment methods for the winter pulse crops in no-till conditions.
- Develop tillage guidelines that assess the sustainability of reduced tillage in irrigated and rain-fed pulse production systems.

- Increase adoption of no-till across the pulse growing regions reducing production costs for producers
- Evaluate the quality, nutrition, and safety of marketed products of various pulse crops from diverse production conditions and environments.
- Determine regionally-specific management strategies to enhance the production and quality of pulse crops
- Enhance systems models and decision aids that evaluate the potential of pulse crop productions for global food security while maintaining soil and environmental quality.

3.2.5.4.2 Long Term (10 years)

- Develop economic risk averting management strategies that improve soil productivity, enhance soil and water conservation and nutrient cycling, and reduce fuel and pesticide use while enhancing the natural resource base.
- Identify optimal rotation sequences that reduce reliance on off-farm inputs to increase the profitability of the production system.

3.2.5.5 Impacts

3.2.5.5.1 Economical

Increased profitability through higher yield, lower input costs .

3.2.5.5.2 Environmental

Reduced soil loss, fossil fuel use, pesticide use, and greenhouse gas emission while improving the sustainability of the cropping system through better water and nutrient use

3.2.5.5.3 Sociological

The ability to develop cropping systems that enhance soil resources would afford growers the opportunity to try alternate crops in rotation with pulses. An expanded cropping options would invariably lead to more successful farming and would enhance the social and economic stability of rural communities.

3.2.5.6 Estimated Cost of Research

We anticipate approximately $ 12 million annually will be required to realize the short, medium and long-term goals of this project.

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3.3 Optimize nitrogen fixation and other microbial interactions.

3.3.1 Develop varieties with improved Nitrogen fixation.

3.3.1.1 Background

The major focus of the pulse breeding programs has been on yield, agronomic and quality improvement. Essential criteria for yield and quality traits are unlikely to change and will continue to be emphasized by breeders. The array of agronomic, disease and phenology traits differ by pulse types but plant architecture, maturity and disease/pest/stress resistance dominate the focus of the breeding program. Neglected among these traits are plant nutrition traits including tolerance to low soil fertility, nutrient use efficiency, and symbiotic nitrogen fixation. Over $100 billion worth of nitrogen fertilizer is used in agriculture production each year and more than half of that does not end up in the plant but instead becomes a pollution source (Ridley, 2009). As growers struggle to optimize yields in an environmentally sustainable fashion, pulse breeders need to consider the genetic enhancement of crops for efficient use of nutrients. Pulses are unique in the plant kingdom in that they have the ability to fix atmospheric nitrogen through the process of symbiosis with *Rhizobium*. Enhancing biological nitrogen fixation (BNF) ability of pulses through breeding and selection would provide growers with option of reducing fertilizer costs and the commensurate potential contamination of ground water. For example bean growers in the Saginaw Valley and thumb of Michigan farm in the Great Lakes water shed and any loss of N through leaching poses problems that require action. Reducing the amount of fertilizer used to produce a successful bean crop would improve land stewardship and avoid potential environmental issues in the future. Fertilizer recommendations for bean production in Michigan range from 44 kg N ha^{-1} at planting to higher rates (66 kg) for narrow row production systems where plant populations are higher. In order to optimize productivity of large seeded kidney and cranberry beans, growers may apply additional N as a side dressing at flowering and rates in excess of 100 kg N are not uncommon. The unfortunate fact is that this practice is common in the central-west part of the state in course textured soils where leaching is a serious problem. In addition studies show that bean plants utilize only 50% of extraneous nitrogen applied as fertilizer (Bliss, 1993). In contrast no additional N is applied to the soybean crop in Michigan as BNF is adequate to meet the plants needs for nutrients and optimum yields.

3.3.1.2 Research Need/Problem Statement

Typically pulse breeding programs supplement soil nitrogen with fertilizer during the process of selection and yield evaluation. As a result, genotypes are not selected for their ability to fix nitrogen while being selected for disease resistance, maturity, and other agronomic traits. Graham (1981) and Bliss (1993) have suggested that selection based on yield under low N soils indirectly permits selection for improved BNF. Yield is a major component of modern breeding programs, and perhaps the primary factor affecting success of a genotype in being considered for commercial release. Expanding testing sites to include a low-N site could provide an opportunity to assess the BNF ability of pulse genotypes. In addition to field testing for BNF, different lab and greenhouse protocols have been tested including N isotope discrimination and acetylene reduction methods where comparisons are made to a non-fixing reference crop (Graham et al., 2003; St. Clair et al., 1988). Non nodulating genotypes are available in some pulse species and these are very valuable in establishing the levels of endogenous nitrogen in the soil in the absence of fixation (Park and Buttery, 2006).

3.3.1.3 Approach/Outline of Research

- To screen elite adapted pulse germplasm for BNF capacity., the initial step in this study will be to assess the range of BNF ability in an elite group of cultivars in all the major commercial pulse classes and advanced lines adapted in the U.S. A suitable greenhouse test would have to be developed for different pulse crops. Breeders will need to identify low N site for field screening and selection can be based on yield under these low N sites, assuming that a non-nodulating genotype is available as a control to determine as soil-N levels. This work would need to be repeated over years and locations to confirm N-fixing potential of different genotypes.
- To develop genetic populations to study inheritance of BNF trait. Once superior N-fixing pulse lines have been identified they would be used as parents and crossed to locally adapted lines. Those populations would be advanced through single seed descent to the F6 generation F4:6 when recombinant inbred lines (RILs) will be available for greenhouse and field testing. Since BNF is a quantitative trait, RIL populations can be replicated over time and locations to estimate heritability and provide phenotypic data for quantitative trait analysis (QTL) analysis.
- To identify molecular markers associated with BNF trait and conduct QTL analysis. RIL populations would be developed and phenotyped in the field and greenhouse for BNF and the same RILs will be genotyped using simple sequence repeat (SSR) markers. The genotypic data will be combined with the phenotypic and a QTL analysis will be conducted to identify those regions associated with improved fixation in pulses. Mapmaker and QTL Cartographer will be used to map these regions to the core map in different pulses for further study and use in future marker-assisted selection (MAS) studies.

3.3.1.4 Outcomes

3.3.1.4.1 Immediate(2 years)

- The short term research would provide information on those pulse varieties and genotypes that are the best N-fixers. This would be useful to growers interested in reducing the use of N in their management programs. It would be of particular interest to organic growers who would not the opportunity to add fertilizer nitrogen.

3.3.1.4.2 Midterm (6 years)

- Progeny developed from crossing to improve N-fixation would be available for testing and initial seed distribution to interested pulse growers. The adaptation of these materials would need to be tested over a broad range of soil types, locations and management practices.

3.3.1.4.3 Long Term (10 years)

- Pulse varieties with enhanced levels of N-fixation would be available to growers and provide the opportunity to rely on N-fixation for their total N needs. Residual N fixed by these high fixing varieties would be available in the soil for next non legume crop and so reduce fertilizer use in subsequent crops.

3.3.1.5 Impacts

3.3.1.5.1 Economical

The estimated cost of N-fertilizer to plant 200,000 acres of dry beans in Michigan in 2010 is $4m [N-rate: 50 pounds per acre at $0.40 per pound~$20/acre]. This figure was closer to $8m in 2007 and 2008 due to the higher cost of oil and natural gas in those years. A 25% reduction in the use of N-fertilizer would be an annual savings to bean growers of $1m. Enhanced levels of BNF in future dry bean varieties would also facilitate the transition in both conventional and organic production practices of dry beans in Michigan, by reducing the need and costs of expensive fertilizer. Similar estimates could be extended to the pea and lentil acreages planted in northern Great Plains and western states.

3.3.1.5.2 Environmental

If enhanced levels of BNF were available to dry bean producers, the reduction in fertilizer use would help reduce environmental issues in the Great Lakes watershed and sustain the productivity of a vital commodity and an important industry in the mid-Michigan area. Similar benefits would apply in other fragile production areas in the U.S. where pulse crops are grown.

3.3.1.5.3 Sociological

Costs of supplying nutrients to sustain crop production are increasing on a worldwide scale. Cultivars that are better able to acquire nutrients from the soil, or fix nitrogen will help reduce costs to producers. The research proposed in this project is to improve pulse crops for enhanced nitrogen fixation. Many pulses are poor N-fixers and require fertilization to produce competitive yields. Genetic variability for enhanced levels of N-fixation exists, but in unadapted germplasm. Genotypes with enhanced fixation will be crossed into adapted local cultivars. Progeny will be phenotyped in greenhouse and field trials and QTL analysis will be conducted on these populations to map genes conditioning N-fixation in different pulse crops. Clearly opportunities exist to enhance BNF in pulses through breeding.

3.3.1.6 Expected Costs of Research

$10 M will be required to realize the short, medium and long-term goals of this project across a number of pulse crops in diverse production/ management systems.

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3.3.2 Identify plant x rhizobia interactions that maximize N Fixation.

3.3.2.1 Background

The increase in global agricultural production that has been realized since the 1940's has largely been the result of the development of improved crop varieties coupled with increased applications of N fertilizer. Unfortunately, the process used for the industrial production of anhydrous ammonia, the most common primary source of N in production agriculture, relies on the use of natural gas to provide N and sophisticated equipment and considerable energy investments to convert natural gas to ammonia (NH_3) and cool the ammonia so that it can be stored as a liquid. In addition, commercially produced ammonia has many other important industrial uses besides fertilizer products, including its use in the manufacture of dyes, pharmaceuticals, plastics, pulp and paper. Agricultural production systems must compete with these other industries for available ammonia. Approximately 90% of the total costs for producing ammonia are associated with the costs of natural gas. Increasing prices for natural gas have been primarily responsible for a nearly 300% increase in the price of nitrogen fertilizer in the US over the past three years. Increasing global demand for natural gas coupled with increased demand for ammonia for agricultural and industrial production in emerging markets suggest that nitrogen fertilizer prices in the US will remain high and likely increase further.

Lentils can fix atmospheric N_2 through an association between the plant and a beneficial soil bacterium (*Rhizobium leguminosarum* bv. *viciae*), which infects the plant roots and resides in root nodules. It has been estimated that US agricultural production costs are annually reduced $7-10 billion through the contribution of N fertilizers produced by rhizobia within legume roots. The fertilizer produced in legume roots, besides reducing production costs, can also serve as an important source of N for organic production systems that cannot use fertilizers produced from industrial processes.

Some pulse crops are more efficient at fixing nitrogen. Early-maturing common bean genotypes only fix 35 kg N ha^{-1} compared to 109 kg N ha^{-1} fixed for later maturing genotypes (Piha and Munns, 1997). Bliss (1993) estimated 50 kg N ha^{-1} fixed by bean, whereas Graham et al. (2003) reported fixation rates among bean genotypes ranging from 0 kg N ha^{-1} to 150 kg N ha^{-1}. Any estimate of bean plants' need for N must be related to productivity. Growers seeking yield(s) 2,500 kg ha^{-1} (state average yield in MI in 2009 was 2090 kg ha^{-1}) would need to apply 100 kg N ha^{-1} either in the form of fertilizer, or be available from soil organic matter, crop rotations and residual N or from fixation. If only 50% of extraneous N is taken up by the bean plant, as the remainder is lost to leaching, optimizing yields will require reliance from other sources among them fixation. Accordingly, beans would have to fix between 50 and 100 kg N ha^{-1} in order to achieve competitive yields without the application of N fertilizer. Farmers are concerned that they will lose yield if they did not fertilize their bean crop adequately. To access potential yield losses, studies (Heilig, 2010) were conducted over a 3-year period (2007-2009) in Michigan, using 32 bean genotypes grown side by side, where one treatment followed conventional practices (50kg N applied at planting) and the second was an organic system where plants had to rely on fixation or residual soil nitrogen from previous legume crop. It could be argued that other factors such as diseases, weeds and pests attributed to yield losses between management systems, but fertility differences played a major role. Yields in organic system averaged 2058 kg ha^{-1} compared to 2507 kg ha^{-1} for conventional equivalent to a difference of 18%. Genotypes ranged in yield from 1377 to 2598 kg ha^{-1} in the organic treatment compared to 1641 to 3385 kg ha^{-1} in the conventional but certain genotypes performed well under both treatments. N-yield (measured as nitrogen concentration in the seed harvested) ranged from 72 kg ha^{-1} in organic compared to 102 kg ha^{-1} under conventional treatment. Data suggest that there is genetic variability for BNF among bean genotypes and BNF could be enhanced if selection was practiced for this trait.

Such a complex biological process as nitrogen fixation by soil bacteria residing in a lentil root is influenced by many factors, both abiotic and biotic. Significant variation has been observed among different isolates of *R. leguminosarum* and different varieties within many species of legumes for the ability to fix N_2. Prior research indicates that both the selection of *R. leguminosarum* inoculant and the genetic makeup of the lentil variety affect the amount of N fixed in this relationship. Unfortunately, very little work has been done on examining the effects of genotype, rhizobium isolate and plant-rhizobium interactions on N_2 fixation using lentil varieties and isolates of *R. leguminosarum* that are common to the pulse production regions of the US. It has long been known that the legume-rhizobial symbiosis is species-specific, but some recent studies have found that this symbiosis may even be cultivar-strain specific. That is, different varieties within the same plant species may prefer different optimal rhizobial strains for maximum N fixation. In several legumes, including pea (*Pisum sativum* L.) (Skot 1983) soybean [*Glycine max* L.(Merr.)] (Danso et al. 1987; Israel 1981) and dry bean (Valverde and Otabbong, 1997) significant plant host, strain, and host x strain interaction effects have been observed on N_2 fixation. Hafeez et al. (2000) examined plant host and rhizobia effects on N_2 fixation in lentils using four rhizobial strains and six lentil varieties. Tests were conducted in field soil that was deficient in N and had a very small indigenous population of *R. leguminosarum* bv. *viciae*. Significant differences among plant genotypes were detected for number of nodules, plant biomass, grain yield and total fixed N. Significant differences among rhizobium isolates were also observed for these four parameters. In addition, significant differences were observed for both plant genotypes and rhizobium isolates for the percent total plant N derived from biological fixation (%P_{fix}) (Hafeez et al. 2000). The range of %P_{fix} for all genotype x rhizobia strain combinations was 3-52%. These results suggest that efforts to maximize N_2 fixation in lentils will be dependent on identifying optimal lentil and rhizobia strain combinations, but could lead to doubling or greater increase in total N_2 fixation.

The bean breeding program in Michigan recently developed a greenhouse test to measure BNF in dry bean (Heilig, 2010). Seed were inoculated with *Rhizobium etli* strain UMR 1597 and the plants were grown on an inert N-free media and vegetative dry weight determined at flowering. In that system a high N-fixing check variety Puebla-152 fixed 109 mg N. Among the 33 genotypes tested, the next highest fixers were the most recent MSU releases. ‘Santa Fe’ pinto fixed 72 mg and ‘Zorro’ black bean fixed 71mg. The widely grown ‘Vista’ navy only fixed 33mg and the old ‘Sanilac’ navy bean known as low N-fixing check (St. Clair et al., 1988) only fixed 20 mg N. Included in the same test was a non- nodulating navy bean R99 (Park and Buttery, 2006) which allowed an estimation of %N fixed from the atmosphere and values varied from 89% for Santa Fe to 59% for Vista.

Vasquez-Arroyo et al. (1998) studied the occupancy of the nodules of three field grown common bean genotypes. They discovered that there was considerable variability in the acetylene reduction values of the different strains isolated and that they had different abilities to compete for nodulation sites. In addition there was a strain x genotype interaction. For example strain N4, as identified in the study had poor acetylene reduction values with common bean genotype

FM-M-38, while the same bean genotype had high acetylene reduction values with strain Q21. In the same study 64% of nodules on the roots of common bean genotype Negro Queretaro were inhabited by *Rhizobium* strain Q21 (Vasquez-Arroyo et al., 1998). Rosas et al. (1998) performed competition studies by mutating *Rhizobium etli* strain KIM5s, creating a non-fixing strain. Plants were planted in the greenhouse in a low nitrogen soil mix containing indigenous *Rhizobium* sp. Pots were inoculated with the mutated strain, called KM6001. Plants were later evaluated visually for color of their foliage. Plants that formed nodules with the non-fixing mutant *Rhizobium* strain would be lighter green since they were fixing less nitrogen, while those that were dark green were nodulated with indigenous strains able to fix nitrogen. Of the 820 genotypes screened, two did not nodulate normally, the navy bean Sanilac and a non-nodulating line NOD125 developed at CIAT. Those common bean genotypes showing N deficiency yellow color preferentially selected in some manner the non-fixing *Rhizobium etli* strain KM6001. By extension the researchers identified common bean genotypes that preferentially nodulated with *Rhizobium etli* strain KIM5s, which is a strain superior at fixing nitrogen. Identifying common bean genotypes which may form associations with specific applied inoculant strains would circumvent the problem of forming nodules with inefficient indigenous strains of *Rhizobium*.

3.3.2.2 Problem Statement/Research Need

Between 2005-2008 the global average cost of nitrogen fertilizer has more than tripled (Fertecon, NYMEX, 2008). Because nitrogen fertilizers are essential for high yield in monocot grain crops and because the price of chemical fertilizers is closely tied to the cost of methane used to manufacture them, recent fluctuations in energy prices have had a serious destabilizing effect on agriculture. Farmers now must anticipate swings in fertilizer prices when calculating yield and profit. This is difficult, not only because energy prices are subject to a large number of production and geopolitical factors, but also because most (70-80%) of the nitrogen fertilizer used in American agriculture is imported. If more nitrogen could be reliably obtained from legume crops in rotation, the value of that locally produced nitrogen would be an internal part of the agricultural system and not an external input.

The efficiency of biological nitrogen fixation in legumes is dependent on several factors, including the strain of Rhizobia, specific crop variety and strain x variety interactions. Interactions between rhizobia strains and specific host plant genotypes need to be identified that will maximize the efficiency of nitrogen fixation in pulse cropping systems.

3.3.2.3 Approach/Outline of Research

Isolates of *Rhizobium leguminosarum* and *Mesorhizobium cicieri* will be obtained from root nodules collected from various pulse growing regions in the US. Nodules will be surface-sterilized and streaked onto yeast extract mannitol agar (YEMA) for isolation of single strains. DNA will be extracted from each strain and subjected to PCR to amplify DNA polymorphisms that can be used to identify genetically distinct isolates.

Lentil, pea, chickpea and dry bean will be grown as individual plants in sterile “conetainers” using 5 replicates per plant line in a low-nutrient sand-based potting mix. Varieties will be chosen so that the highest yielding and most commercially accepted varieties for different production regions are included in the analysis. For each species of pulse crop, at least 10 different varieties will be examined. Each container will be lightly fertilized. with 10 ml 2 M ammonium sulfate with 10% isotopic 15-N at 1 wk and 2 wk to isotopically “label” the plant biomass. For each rhizobia isolate, each conetainer will receive 5 ml total volume of a broth culture of consisting of 105 – 106 cells per ml at 3 wk and 6 wk after planting. Plants will be harvested at 12 wk after planting. Biomass will be dried, weighed, ground, and analyzed for both total N and 15-N. The proportion and total amount of plant N that was fixed by the rhizobial symbiont will be determined with this information. The effect of plant host genotype, rhizobia isolate and host genotype x rhizobia isolate interactions on total nitrogen fixation will be determined through an analysis of variance (ANOVA). Experiments will then be conducted in several different field locations to examine if the results obtained from greenhouse experiments can be replicated under production agriculture conditions.

3.3.2.4 Outcomes

3.3.2.4.1 Immediate(2 years)

- Varieties of pea, lentil, chickpea and dry bean will be identified that are superior in their ability to accumulate nitrogen through biological fixation when inoculated with a range of rhizobia isolates.
- Rhizobia isolates (both R. leguminosarum and M. ciceri) will be identified that are superior in their ability to fix nitrogen when used to inoculate a range of pulse host varieties.
- Specific plant host x rhizobia isolate interaction will be identified that result in the highest levels of biological nitrogen fixation.

3.3.2.4.2 Midterm (6 years)

- Isolates of rhizobia will be identified that are superior in their ability to fix nitrogen when used to inoculate particular market classes of peas, lentils, chickpeas and dry bean.
- The relationship will be determined between the number and size of root nodules and pulse crop grain yield and quality.
- Science-based recommendations will be made available to growers regarding the optimal choice of rhizobium isolate to use for inoculating specific pulse varieties grown in specific production regions.

3.3.2.4.3 Long Term (10 years)

- Enhanced production of nitrogen through biological fixation will reduce costs associated with the application of exogenous nitrogen fertilizer for small grain growers that use pulse crops in their rotations.
- More acres used to grow small grains will be planted with pulses as rotational crops due to the economic benefit conferred to these growers through biological nitrogen fixation.

3.3.2.5 Impacts

3.3.2.5.1 Economical

We anticipate that the identification of specific rhizobia strain x pulse variety interactions that result in increased fixation of biological nitrogen will result in a considerable expansion of pulse crop production in the US. This will also reduce the amount of exogenous nitrogen fertilizer required to sustain small grain production in the US, which should increase profitability and competitiveness for US wheat and barley growers.

3.3.2.5.2 Environmental

Realizing the objectives of this research program will result in an increase in the acreage of pulse crops produced in the US. This will result in a reduction in fertilizer, pesticide and herbicide usage in these areas of cultivation, as pulse crops typically require very little inputs of these agrochemicals as compared to other crops. A greater proportion of total nitrogen fertilizer requirements for small grains such as wheat and barley will come from biological nitrogen fixation. This will reduce the mechanical requirements of the small grain industry that are associated with the application of nitrogen fertilizer, which will result in reduced greenhouse gas emissions from the burning of gas and diesel fuel to power tractors. Less use of tractors in small grain production will also reduce soil compaction and erosion in the grain producing regions of the US.

3.3.2.5.3 Sociological

Increased production of pulse crops due to economic and environmental benefits conferred through their ability to more efficiently fix nitrogen will enhance the economic viability of American rural communities in which these crops are grown. Chemically produced nitrogen fertilizer is a globally traded commodity. The security of American small grain agricultural production will be enhanced by reducing its reliance on chemically synthesized nitrogen that is often produced in other nations.

3.3.2.6 Expected Costs of Research

We anticipate approximately $15 M will be required to realize the short, medium and long-term goals of this project.

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3.3.3 Characterize and maintain rhizobia collection.

3.3.3.1 Background

Rhizobial bacteria living in symbiotic association with pulses are necessary for nitrogen fixation in these plants. It is known that different species and strains of these bacteria have different abilities to associate with various plants, and to effectively supply nitrogen. In pea (*Pisum sativum* L.) (Skot 1983), soybean [*Glycine max* L.(Merr.)] (Danso et al. 1987; Israel 1981) and dry bean (*Phaseolus vulgaris* L.) (Valverde and Otabbong, 1997) significant plant genotype, strain genotype, and host genotype x strain genotype interaction effects have been observed on N_2 fixation. Hafeez et al. (2000) examined plant host and rhizobia effects on N_2 fixation in lentils using four rhizobial strains and six lentil varieties. Significant differences among rhizobium isolates were detected for number of nodules, plant biomass, grain yield and total fixed N.

Recently, Abi-Ghanem et al. (2009) examined the effects of rhizobium strain on N_2 fixation in lentil cultivars grown in the US. Five lentil cultivars (Eston, Meritt, Pennell, Pardina and Riveland) and 13 *R. leguminosarum* bv. *viciae* strains from commercial sources were examined. Rhizobium strain effects on the number of nodules/plant were not significant. Significant rhizobia strain effects were observed for percent total plant N derived from biological fixation ($\%P_{fix}$). The $\%P_{fix}$ was also influenced by interactions between plant genotype and rhizobia strains. Eston and Meritt had $\%P_{fix} > 70$ for inoculations with all 13 strains, while for Riveland $\%P_{fix} > 70$ were observed for only 8 of 13 strains. These results suggest that genetic differences among rhizobia strains can affect their ability to efficiently fix nitrogen in different pulse host genotypes.

The results of Hafeez et al (2000) and Abi-Ghanem et al. (2009) are robust with respect to the effect of plant genotype on nitrogen fixation, but their results regarding rhizobium strain effects and plant genotype x rhizobium strain interactions must be accepted with caution. This is because in both studies, there was no definitive proof provided to support the assumption that the strains used were genetically different. This information would be critical in assessing relatedness between indigenous *R. leguminosarum* bv. *viciae* strains and strains present in commercial inoculant preparations. At the present it is not well understood how commercial inoculants compete with indigenous *R. leguminosarum* bv. *viciae* strains. Examples have been reported of both the inability of commercial inoculants to compete with indigenous strains (Moawad et al. 1998) and the successful competitive ability of commercial inoculants (Shah et al. 2002). Besides commercially available strains, very little is also known about the genetic diversity among wild isolates of rhizobia. Greater efforts to collect rhizobia from wild legumes and landraces of food legumes could result in the isolation of strains that are more efficient at fixing nitrogen than currently available commercial strains. In addition to the lack of knowledge concerning genetic variation among regional, national, or global populations of *R. leguminosarum* bv. *viciae*, nothing is known about genetic variation among isolates of *Mesorhizobium ciceri*, the only rhizobia species that can efficiently fix nitrogen in chickpeas.

In order to increase biological nitrogen fixation in pulses, produce pulses in a wide range of environmental conditions, and maximize energy efficiency, the global genetic resource of these bacteria must be available, understood, and better utilized. Better genetic characterization of the global rhizobia population will help reduce costs to American small grain growers associated with the use of chemically synthesized nitrogen fertilizer. Specific strains of rhizobia can be unambiguously identified that can fix nitrogen better than other strains when used to inoculate particular plant varieties in a given environment.

3.3.3.2 Problem Statement/Research Need

How much genetic variation exists among regional, national, and global populations of *R. leguminoasrum* and *M. ciceri* is poorly understood. A better characterization of genetic diversity among these populations could lead to an improved understanding of rhizobia strain effects on the efficiency of nitrogen fixation in pulse crops. This could result in the identification of specific strain x plant x environment combinations that maximize biological nitrogen fixation.

Greater efforts to collect rhizobia from wild legumes and landraces of food legumes could result in the isolation of strains that are more efficient at fixing nitrogen than currently available commercial strains. The objectives of this research are to collect rhizobium isolates from wild legumes and land races of grain legumes and to determine how genetically diverse is the global rhizobia population. To facilitate availability of this important global resource, a “core collection” is isolates which represents the genetic diversity among the global rhizobia population will have to be identified and maintained. Collect, maintain, characterize, and make available for research and commerce a broad variety of rhizobial bacteria.

3.3.3.3 Approach/Outline of Research

Isolates of *Rhizobium leguminosarum* and *Mesorhizobium cicieri* will be obtained from root nodules collected from various pulse growing regions in the US and other pulse producing nations. Nodules will be collected from varieties and landraces of pea, lentil, chickpea and dry bean and also from Nodules will also be isolated from other *Phaseolus* sp., *Pisum* sp., *Lens* sp. and *Cicer* sp. collected as wild plants. Nodules will be surface-sterilized and streaked onto yeast extract mannitol agar (YEMA) for isolation of single strains.

DNA will be extracted from these isolates using the FastPrep DNA extraction kit according to manufacturer’s recommendations. DNA will be quantified by fluorometry and diluted for use in PCR reactions. Genetic diversity among isolates will be examined by two methods. First, DNA from each isolate will be amplified using arbitrary sequence related amplified polymorphisms (SRAP) markers (Li and Quiroz, 2001). Genetic similarities between isolates will be determined (Nei and Li, 1972) and a dendrogram representing the genetic relationships among isolates will be generated based on the unweighted pair-group method using arithmetic averages (UPGMA) cluster analysis. In addition, nearly full length 16S rRNA will be amplified from 1-2 representatives of each unique SRAP using the primers Y1 and Y3 (Young et al. 1991; Laranjo et al. 2004). The DNA sequences of the 16S rRNA amplicons will be determined for each isolate and phylogenies will be determined by the maximum likelihood method. These analyses are needed to develop a long-term collection of unique strains which will be stored in duplicate in 10% glycerol at -80C.

3.3.3.4 Outcomes

3.3.3.4.1 Immediate(2 years)

- Improved understanding of genetic diversity among commercially available rhizobia isolates.

- Increase the size of the collection of rhizobia isolates (both R. leguminosarum and M. ciceri) and the diversity of host crops and geographic regions represented by the collection.

3.3.3.4.2 Midterm (6 years)

- A 'core' collection of rhizobia isolates will be developed, maintained, and made globally available.
- Novel rhizobia isolates will be identified from wild legumes and landraces of grain legumes that are superior to currently available commercial isolates in their ability to fix nitrogen in pulse crops.
- Rhizobia isolates will be identified that have superior survival and nitrogen fixing efficiency in specific production environments.
- Improved understanding of how rhizobia strain genotype x plant genotype x environment interactions affect the efficiency of nitrogen fixation.

3.3.3.4.3 Long Term (10 years)

- Enhanced production of nitrogen through biological fixation will reduce costs associated with the application of exogenous nitrogen fertilizer for small grain growers that use pulse crops in their rotations.
- More acres used to grow small grains will be planted with pulses as rotational crops due to the economic benefit conferred to these growers through biological nitrogen fixation.
- Global food security will be improved through increased production of pulse crops and a reduction in costs associated with the application of chemically synthesized nitrogen fertilizer will be realized for small grain growers.

3.3.3.5 Impacts

3.3.3.5.1 Economical

A more comprehensive understanding of the genetic structure of the global rhizobia population will allow for the identification of specific strains that are superior in their ability to fix nitrogen in particular pulse species and varieties. This will increase the ability to produce nitrogen fertilizer for small grains through biological fixation in pulse crops, which will reduce fertilizer costs for small grain growers. This should increase the profitability and competitiveness of US wheat and barley growers.

3.3.3.5.2 Environmental

A greater proportion of total nitrogen fertilizer requirements for small grains such as wheat and barley will come from biological nitrogen fixation. This will reduce the mechanical requirements of the small grain industry that are associated with the application of nitrogen fertilizer, resulting in reduced greenhouse gas emissions from the burning of gas and diesel fuel to power tractors. Less use of tractors in small grain production will also reduce soil compaction and erosion in the grain producing regions of the US.

3.3.3.5.3 Sociological

Chemically produced nitrogen fertilizer is a globally traded commodity. The security of American small grain agricultural production will be enhanced by reducing its reliance on chemically synthesized nitrogen that is often produced in other nations. Specific rhizobia isolates will be identified that can fix nitrogen in marginal soils and climates, which will expand the global area that can be used to successfully produce pulse crops. This will enhance both global food security and overall human health through increased nutritional benefits conferred by the consumption of pulse crops

3.3.3.6 Expected Costs of Research

We anticipate approximately $10 M will be required to realize the short, medium and long-term goals of this project.

3.3.3.7 Bibliography

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