David R. Holding (PhD), Assistant Professor, Department of Agronomy and Horticulture and Center for Plant Science Innovation, University of Nebraska Lincoln, E323 Beadle Center, Lincoln, NE, 68588, Tel.: (402) 472-1357, Fax: (402) 472-3139, e-mail: dholding2@unl.edu

Summary of Current Research (78%) and Teaching (20%) (2% service)

- Investigating the nature, mode of action and potential applications of *opaque2* modifier genes in *Quality Protein Maize* using transcriptomic and proteomic profiling as well as biotechnological approaches
- Studying endosperm maturation and protein quality using maize and sorghum opaque endosperm mutants and functionally characterizing the gene products
- Seed and whole plant functional genomics of maize and sorghum using gamma-radiation induced mutants and high throughput mapping, DNA and RNA sequencing and proteomic profiling
- Breeding of *Quality Protein Popcorn* with ConAgra foods
- Teaching Agro 131, Plant Science. Lead role in innovating instruction in basic physiology, anatomy, biochemistry, genetics and biotechnology of crop and model plants. Co-author of electronic Text Book, "Plant Science: The biology of plants grown for a purpose"

A. Education

University of Sussex, UK	Biochemistry	BSc, 1991
Kings College London, UK	Plant Molecular Genetics	PhD, 1997

B. Professional Appointments

Assistant Professor	University of Nebraska Jan 2009-present	Department Agronomy and Horticulture & invited full member of Center for Plant Science Innovation
Postdoctoral	University of Arizona, 2002-2008	Department of Plant Sciences Functional genomics of maize endosperm formation and protein quality
Postdoctoral	UC Riverside, 1997-2001	Department of Botany and Plant Sciences Studied meristem regulation, vascular patterning and endosperm development in *Arabidopsis thaliana*

C. Peer reviewed publications

***** work performed at UNL
****** work performed at UNL, corresponding author
(for UNL papers, Holding lab contributed 100% unless otherwise noted)

*Gelli, M., Dou, Y., Konda, A.R., Zhang, C., **Holding, D.R.,** and Dweikat, I. (2014). Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. BMC Genomics. 15: 179. (Designed experiments and supervised graduate student on 75% of work, co-wrote paper. Dweikat provided genetic materials and funding)

******Yuan, L., Dou, Y., Kianian, S., Zhang, C. and **Holding, D.R**. (2014) Deletion mutagenesis identifies a haploinsufficient role for gamma-zein in *opaque-2* endosperm modification. Plant Physiol. 164: 119-130.

****Holding, D.R.** (2014). Recent advances in the study of prolamin storage protein organization and function. Frontiers in Plant Science. Special issue: Advances in Seed Biology, 5:276. doi: 10.3389/fpls.2014.00276. Combined review and original research paper.

*****Wu, Y., Yuan, L., Guo, X., **Holding, D.R.**, and Messing, J. (2013). Mutation Causing a Single Amino Acid Substitution Creates High Food Value Trait in Sorghum. Nature Communications, Volume 4, Published 8-16-2013. (performed 50% of work, co-wrote paper)

******Guo, X, Yuan, L, Chen, H, Sato, S.J., Clemente, T.E., and **Holding, D.R.** (2013). Non-redundant function of zeins and their correct stoichiometric ratio drive protein body formation in maize endosperm. Plant Physiol. **162**, 1359–1369

***Holding, D.R.**, and Messing, J. (2013) Evolution, structure and function of prolamin storage proteins. Invited, peer reviewed book chapter *in*: Seed Genomics, First edition, Wiley-Blackwell Publishing, Editor, Becraft, P. *pp138-158*

******Guo X., Ronhovde K.J., Yuan L., Yao B., Soundararajan M.P., Elthon T.E., Zhang C., **Holding D.R. (2012)** Pyrophosphate dependent fructose-6-phosphate 1-phosphotransferase induction and attenuation of Hsp gene expression during endosperm modification in Quality Protein Maize. Plant Physiol. *158: 917-929*

***Holding, D.R.,** Hunter, B.G., Klingler, J.P., Wu, S., Guo, X., Gibbon, B.C., Wu, R., Schulze, J., Jung, R., and Larkins, B.A. (2011) Characterization of *opaque2* modifier QTLs and candidate genes in recombinant inbred lines derived from the K0326Y Quality Protein Maize inbred. Theoretical and Applied Genetics 122, 783-794 (performed 60% of work while at UNL, wrote paper)

*Reyes, F.C., Chung, T., **Holding, D.**, Jung, R., Vierstra, R., and Otegui, M.S. (2011). Delivery of Prolamins to the Protein Storage Vacuole in Maize Aleurone Cells, The Plant Cell 23, 769-784. (designed and performed 20% of work at UNL)

****Holding, D.R.**, Meeley, R.B., Hazebroek, J., Selinger, D, Jung, R. and Larkins, B.A. (2010) Identification and characterization of the maize arogenate dehydrogenase gene family, Journal of Experimental Botany 61: 3663-3673.

*Wu, R., **Holding, D.R.**, and Messing, J. (2010) Gamma zeins are essential for endosperm modification in Quality Protein Maize. Proceedings of the National Academy of Sciences, USA. 107: 12,810-12,815. (contributed research materials, evaluated data, co-wrote paper)

***Holding, D.R.** and Larkins, B. A. (2009) Zein storage proteins, Chapter V. Invited, peer reviewed book chapter in: Molecular Biology and Physical Studies, in Molecular Genetic Approaches to Maize Improvement, (A.L. Kriz and B.A. Larkins eds.) pp 269-286. Springer-Verlag Publishers, Heidelberg, Germany. (wrote paper)

Holding, D.R., Hunter, B.G., Chung, T., Gibbon, B.C., Ford, C.F., Bharti, A.K., Messing, J., Hamaker, B.R. and Larkins, B.A. (2008) Genetic Analysis of *opaque2* Modifier Loci in Quality Protein Maize. Theoretical and applied genetics 117: 157-170.

Holding, D.R. and Larkins, B.A. (2008) Genetic Modification of Seed Storage Proteins. Invited peer reviewed chapter in: Advances in Plant Biochemistry and Molecular Biology (Lewis, N.G., Ed.-in-chief) Vol. 1, Bioengineering and Molecular Biology of Plant Pathways (Bohnert, H.J. and Nguyen, H.T, eds.), pp. 107-133. Elsevier Publishers, Oxford, UK.

Holding, D.R, Otegui, M.S., Li, B., Meeley, R.B., Hunter, B.G., Jung. R. and Larkins, B.A. (2007) The maize Floury1 gene encodes a novel ER protein involved in zein protein-body formation. The Plant Cell 19:2569-2582.

Settles, M., **Holding, D.**, Tan, B.C. Latshaw, S., Susuki, M, O'Brien, B., Fajardo, D., Wroclwaska, E., Lai, J., Hunter, C., Avigne, W., Peacock, S., Baier, J., Lonon, D., Messing, J., Hannah, L.C., Koch, K., Becraft,

P., Larkins, B., and McCarty, D. (2007) Maize Sequence Indexed Knockouts using the UniformMu Transposon Tagging Population. (2007) BMC Genomics, 8:116.

Holding, D.R. and Larkins, B.A. (2006) The development and importance of zein protein bodies in maize endosperm. Maydica 51 (2): 243-254.

Lopez-Valenzuela, J.A., Gibbon, B.C., **Holding, D.R.**, and Larkins, B.A. (2004) Cytoskeletal proteins are coordinately increased in maize genotypes with high levels of eEF1A. Plant Physiology 135: 1784-1797.

Holding, D.R. and Springer, P.S. (2002) The VASCULAR PREPATTERN enhancer trap marks early vascular development in Arabidopsis. Genesis 33 (4): 155-159 2002.

Holding, D.R. and Springer, P.S. (2002) The Arabidopsis gene PROLIFERA is expressed at all stages of reproductive development and is required for cytokinesis. Planta, 214 373-382.

Springer, P.S., **Holding. D.R**., Groover, A., Yordan, C. and Martienssen, R.A. (2000) The essential Mcm7 protein PROLIFERA is localized to the nucleus of dividing cells during the G1 phase and is required maternally for early Arabidopsis development. Development 127, 1815-1822.

Holding, D.R., Springer, P.S. and Coomber, S.A. (2000) The chloroplast and leaf developmental mutant, pale cress, exhibits light-conditional severity and symptoms characteristic of its ABA deficiency. Annals of Botany. 86, 953-962.

Holding, D.R., McKenzie, R.J. and Coomber, S.A. (1994). Genetic and Structural analysis of five mutants with abnormal root morphology generated by the seed transformation method. Annals of Botany 74, 193-204.

D. Synergistic Activities

- Invited reviewer for multiple international journals (~2 invitations accepted per month)
- Reviewer for NSF proposals and USDA research plans
- Advisor for four current PhD students, and two post-doctoral fellows

E. Funding history

Awarded (total **$1,154,020** of which $1,115,020 is awarded to and managed by Holding)

1. Gene discovery for sorghum response to nitrogen. United Sorghum Checkoff Foundation, project period, 03/20/2010 to 03/19/2011, **$39,000** (co-PI with Ismail Dweikat who managed funds).
2. Development of Quality Protein Popcorn as a non-GMO approach to enhanced nutritional quality, pop volume and flavor profile. ConAgra Foods, 9/1/2013 to 8/31/2018, **$532,035** (PI, Oscar Rodriguez and Devin Rose, co-PIs. PI currently assigned all funds)
3. A novel functional genomics platform for dissecting maize kernel maturation and protein quality. USDA AFRI, 11/1/2013 to 10-31-2016, **$412,985** (PI with Chi Zhang as co-PI. PI currently assigned all funds)
4. Engineering high protein quality and high digestibility sorghum through a novel non-GMO functional genomics platform. Externally reviewed, competitive, Center for Plant Science Innovation Seed Grant, 4/1/2014 to 3/31/2016, **$120,000** (PI, Chi Zhang and Ismail Dweikat, co-PIs. PI currently assigned all funds)
5. ARD bridge proposal to provide critical data for NSF PGRP proposal "An integrated functional genomics and proteomics platform for dissecting maize seed composition and size." Internal ARD award **$50,000** 6/1/2014 to 5/31/2015 (PI assigned all funds)

Pending

1. An integrated functional genomics and proteomics platform for dissecting maize seed composition and size. NSF Plant Genome Research Program **$1,471,022**, 01/01/2015 to 12-31-2018

F. Awards

2013 Agricultural Research Division, UNL Institute of Agriculture and Natural Resources 'Junior Faculty Recognition for Excellence in Research'

Summary of Accomplishments

Research – 78% position responsibility (2009-2014)

1. Research grants totaling $1,154,020 (page 15)
   a) Federal funding: $412,985
   b) Industrial funding: $532,035
   c) Foundation funding: $39,000
   d) Internal UNL funding: $170,000

2. 12 peer reviewed publications since 2009 arising from work at UNL (page 15-16)
   a) Nine original journal research papers
   b) Two invited book chapters
   c) One invited journal review

3. 546 total citations and an h-Index of 12 (page 15)

4. 16 research presentations (page 17)
   a) ten conference presentations
   b) six invited external oral presentations

5. Main advisor to three PhD students (graduating 2014, 2015, 2017-18), co-advisor to one PhD student (graduated 2013), co-advisor to one MSc student (graduating 2016-17) (page 21)

6. Leading projects in three major interrelated research fields (pages 7-14)

   a) Maize kernel maturation and protein quality
      I) Investigating the nature, mode of action and potential applications of *opaque2* modifier genes in Quality Protein Maize using transcriptomic and proteomic profiling as well as biotechnological approaches
      II) Studying endosperm maturation and protein quality using natural and RNA interference induced opaque endosperm mutants
      III) Functional genomics of maize seed development using gamma-radiation induced mutants and high throughput mapping, DNA and RNA sequencing and proteomic profiling

   b) Sorghum grain digestibility and protein quality
      I) Characterized molecular identity of the *High Digestibility High Lysine* sorghum variant
      II) Creation of novel kafirin variants for improved digestibility and protein quality and sorghum functional genomics using gamma-radiation mutagenesis and high throughput mapping, DNA and RNA sequencing and proteomic profiling

   c) Breeding of *Quality Protein Popcorn* with ConAgra foods

Teaching – 20% position responsibility (2009-2014) (page 18-23)

Team teach Plant Science (Agro 131)

   a) Teach all lectures in two out of four units (driving plant growth and controlling plant growth) for main 150 section (120-160 students), fall and spring 2010-12, fall 2012-13

   b) Provide teaching materials (slides, art work, problems and exams) used by instructors in other sections

   c) Co-author of currently used on-line electronic text-book "Plant Science: The biology of plants grown for a purpose"

Service – 2% position responsibility (2009-2014) (page 24)

Candidate statement

A vital part of maize seed development is the formation of a vitreous (hard) endosperm since it is fundamental to agronomically useful grain characteristics including yield, resistance to insect and fungal damage, resilience during harvest and storage, and appropriate processing characteristics. Paradoxically, grain hardness is usually inversely proportional to grain protein quality. This is because the highly abundant zein storage proteins, that dominate the total seed protein and allow vitreous endosperm formation, are devoid of two essential amino acids, lysine and tryptophan. However, the existence of Quality Protein Maize (QPM) varieties that have high protein quality while maintaining hard kernel texture, illustrate the scope for maize improvement both for human nutrition and improved livestock feeding efficiency. My research focuses broadly on understanding the complex relationship between vitreous grain formation and protein quality in the context of high-lysine QPM varieties as well as in normal maize and sorghum. The research is both basic and applied. On the basic side, by determining the molecular genetic and biochemical processes that affect kernel maturation and protein quality, we can ultimately provide innovative routes to grain improvement. On the applied side, our work is developing new varieties that can be used directly for improved human and livestock nutrition without being subject to regulatory processes applied to genetically modified crops. This is exemplified in the following applied projects that can create tangible outputs in the relatively near future.

1) In addition to the basic seed functional genomics research afforded by our populations of maize deletion mutants, we are identifying novel low-zein variants that have improved lysine content and digestibility. Highly digestible corn varieties have recently been shown to have substantial potential for increased milk production in dairy cattle.

2) Low protein quality is an equally significant problem in sorghum, a crop of increasing importance for marginal land growth in the U.S. and a staple grain in many developing countries. Compounding this is the fact that sorghum grain proteins are less digestible than those of maize. We are making inroads into improving sorghum through our characterization of the molecular basis of the *high digestibility high lysine* sorghum variant as well as creating new low-kafirin deletion lines with which we are developing a partnership with ICRASAT in India for rapid introduction of improved varieties.

3) While the current agro-economic climate is not favorable for introduction of new non-GM <u>field</u> corn varieties with improved output traits (such as nutritional quality), a substantial opportunity exists for popcorn in which company policy and consumer preference is presently dictating a complete avoidance of GM traits. Consequently, since the inception of the transfer of the ConAgra Foods popcorn breeding program to UNL, we have begun a new breeding program in which we are introgressing the high-lysine, vitreous endosperm characteristics of QPM into elite ConAgra popcorn varieties. Popcorn sales have waned in recent years, and improved nutritional quality is one potential way to invigorate and revitalize the market, and bolster the emerging partnership between UNL and ConAgra.

My broad knowledge of vegetative and reproductive growth and development in both model and crop plants and especially my specialization in genetic, biochemical, genomic and biotechnological studies relating to seed biology, has equipped me with a unique skill set and perspective for teaching core concepts in plant science. I introduce key concepts in the mechanisms driving and controlling plant growth with special focus on seed development and crops of economic importance to Nebraska. I take advantage of many opportunities in my teaching of Agro131 to draw on basic concepts from my current research on seed development as well as my past research on vegetative aspects of plant growth from root development to meristem regulation, leaf initiation and vascular patterning. I am fulfilling my goal of enlightening students to a world of opportunities which exist in a career in Plant Sciences. My most treasured and inspiring student evaluation comment so far is:

"Dr. Holding taught me how a plant works!"

RESEARCH (78%)

Research Philosophy and Goals

My research career is driven by three overarching personal philosophies.

The first it that I seek to enhance basic and fundamental knowledge of plant growth and development, especially in relation to seeds, man's most important food source. From the inception of my science learning, I have been fascinated by the way plants grow and respond to environmental cues in their provision of life sustaining food. My deep appreciation and understanding resulted in enthusiasm to share the marvels of plant biology with others, as well as a drive to enhance our basic knowledge. Inspirational sharing and continued acquisition of basic knowledge of model and crop plants is the bedrock of my research and I believe, the bedrock of any university department engaged in plant research. Despite the highly diverse and applied nature work in the UNL Department of Agronomy and Horticulture, our research, teaching and extension all depend on basic knowledge and continued expansion of basic knowledge of plant biology. This is a central theme in my research and teaching.

The second guiding philosophy has been a desire to maximize my impact in science by focusing my interests on crops and problems that potentially can have the largest gains for the human race. Moving towards this ideal involved a gradual transition from studying molecular genetic mechanisms governing root and leaf development in the model plant, *Arabidopsis thaliana*, to studying endosperm formation during Arabidopsis, and later maize, seed development. My switching to maize as a post doc prepared me to take advantage of more readily available funding resources for applied research, at a time when funding for pure basic plant research was rapidly dwindling. Moreover, it allowed me to focus on grain nutritional quality, which is central to the third guiding philosophy.

The third philosophical theme that drives my long-term research goals relates to my belief that the human race needs to adopt more sustainable practices in the way we feed ourselves and our livestock. We are currently on an unsustainable trajectory in which there is increasing global consumption of animal protein as developing countries become more westernized. Paradoxically, while we get closer to maximal yield potential of our crops, negative environmental challenges, such as water shortage and extreme weather events suggest we are approaching a major shortfall in global food supply as early the middle part of the 21st century. In addition to growing our crops as efficiently as possible, increasing our feeding efficiency could help us to meet the challenges we face. Cereal grains such as QPM, that are bred to be more complete protein sources, can be used for livestock feed while reducing the amount of supplementation required. Where maize and sorghum are used for staple human nutrition, high-lysine varieties help in the prevention of protein malnutrition when other protein sources are limited. In developed countries there is growing recognition that diets rich in plant protein and lower in animal protein are healthier and thus, grain sources of high quality plant protein are steadily gaining in popularity.

Summary of current major research projects

1) Maize kernel maturation and protein quality
   - Investigating the nature, mode of action and potential applications of *opaque2* modifier genes in Quality Protein Maize using transcriptomic and proteomic profiling as well as biotechnological approaches
   - Studying endosperm maturation and protein quality using natural and RNA interference induced opaque endosperm mutants
   - Functional genomics of maize seed development using gamma-radiation induced mutants and high throughput mapping, DNA and RNA sequencing and proteomic profiling
2) Sorghum grain digestibility and protein quality
   - Characterized molecular identity of the *High Digestibility High Lysine* sorghum variant
   - Creation of novel kafirin variants for improved digestibility and protein quality and sorghum functional genomics using gamma-radiation mutagenesis and high throughput mapping, DNA and RNA sequencing and proteomic profiling
3) Breeding of *Quality Protein Popcorn*

Relevance of research to department, institute, and university mission and goals

"The role of the University of Nebraska–Lincoln as the primary intellectual and cultural resource for the State is fulfilled through the three missions of the University: teaching, research, and service." My overall role at UNL fits squarely within these three university mission areas. Individual research programs at UNL must balance their contribution to basic scientific knowledge and global improvements for humanity with their direct ability to benefit the Department, Institute, University and State. My diverse research focusing on basic aspects of maize and sorghum kernel development and how these relate to grain texture, protein quality and digestibility, continuously seeks to be, and succeeds in being, relevant in all the above UNL mission areas.

In line with the mission of the Department of Agronomy & Horticulture, as outlined in the preceding sections my research clearly does **"advance the knowledge, theory, and application of plant sciences…to improve the quality of life for citizens of Nebraska and the world"**. With the exception of faculty who are born and bred Nebraskans, and whose entire career progression has been shaped by economic driving forces of the State of Nebraska, all new UNL faculty must adapt their motives and apply their skillset to address the economic needs of our state. I do this by focusing on improving crops of significance to the Nebraska economy.

Nebraska is the third largest producer of corn in the U.S. and the largest producer of popcorn. The latter fact is central to the choice by ConAgra to move their breeding of popcorn to UNL. Any improvements we make to popcorn breeding, either in a general sense through modernizing popcorn genomic breeding capabilities and improvements in neglected agronomic traits or to specific characteristics such as protein quality, will reap economic dividends, for our department, institute, university and State. Ranked number six in acreage, Nebraska is a second tier sorghum producer after Kansas and Texas. However, sorghum has excellent potential for expanded use, especially in the drier western parts of Nebraska. Although I am relatively new to sorghum research, I am enthusiastic about the advantages it offers as a drought resistant crop with excellent value as a feedstock and fuel stock and promising potential for human nutrition, especially as a gluten free wheat alternative. In the words of Jack Harlan, 1971, because of its wide uses and adaptation "sorghum is one of the really indispensable crops required for the survival of humankind". From a scientific perspective, sorghum has some key advantages over maize for research since its fully sequenced genome is not polyploid and is one third of the size of the maize genome. In view of these

agronomic and research advantages, I firmly believe Nebraska should be at the forefront of sorghum research.

The vision of the UNL Institute of Agriculture and Natural Resources (IANR) is that its departments and faculty **"serve Nebraska by providing internationally-recognized science and education to assure the state's competitiveness in a changing world.**" The vision is enacted by **"achieving world-class excellence in: the life sciences, ranging from molecular to global systems; sustainable food, fiber and natural resource systems that support a bio-based economy; economics and environments for a sustainable future…."** The IANR recognizes the need to be at the cutting edge of scientific innovation in the plant sciences both in research where applications are immediate and in areas where the linkage between knowledge and agronomic gain are less direct. Part of my work falls into the latter category, such as understanding the basic biology behind cereal endosperm texture and packaging of storage proteins, as well as developing and promoting seed functional genomics resources for the national and international maize communities. Basic scientific innovation is critical to all crop improvement and the IANR confidently supports this type of work by its departments and faculty.

The Agricultural Research Division (ARD) of IANR showed this confidence by supporting cross disciplinary translational projects with seed grants to selected members of the Center for Plant Science Innovation. I was awarded one of these externally reviewed competitive grants. As a further demonstration of the confidence of ARD and IANR in my work, I was awarded the 2013 "Junior Faculty Recognition for Excellence in research and potential as a Scientist"

Current research projects in detail

Major Research Area 1. Maize kernel maturation and protein quality

a) Investigating the nature, mode of action and potential applications of *opaque2* modifier genes in Quality Protein Maize using transcriptomic and proteomic profiling as well as biotechnological approaches

I) Background

Quality Protein Maize (QPM) was bred from the chalky kernel *opaque-2* (*o2*) mutant that accumulates low levels lysine-devoid, zein storage proteins and thus, higher levels of non-zeins that substantially raise the level of lysine and tryptophan, the two most limiting amino acids in maize and other cereal grains. During breeding of QPM, the essential hard, vitreous endosperm was reintroduced while maintaining the complete protein status of *o2*. QPM inbreds and hybrids have been developed for many climates and are substantially improving both human and livestock nutrition, but their value has yet to be exploited in the developed world. This is partly because the modification process from soft *o2* to QPM is laborious and complicated since it involves multiple Quantitative Trait Loci (QTLs) and the maintenance of the homozygous *o2* allele. It is also partly because protein quality has been a low priority for large seed companies whose primary interest has been yield. At a time when we need to invigorate our utilization of smaller scale, more nutritious grain varieties for natural and non-GM markets, my work on this project seeks to develop a more complete understanding of the molecular genetic basis of QPM. By identifying the genes involved, we gain convenient markers for further development of high protein quality traits in maize.

II) Achievements

We know through various mapping experiments, in different labs, that there are multiple QTLs for QPM that have varying degrees of overlap in different QPM populations. We used microarray and later, Illumina RNA-sequencing (RNA-seq) and proteomics to identify differentially expressed genes that

correspond to these QTLs and this was published in *Theoretical and Applied Genetics* in 2011. The most significant of these genes encodes a major adaptive glycolytic enzyme, Pyrophosphate-dependent Phosphofructokinase (PFP), which is normally expressed at a low level in seeds but is massively induced in QPM seeds. In 2012, we published our model in *Plant Physiology* describing how this enzyme participates in an amelioration of energy deficiency in the maize endosperm that is induced in *o2* and alleviated in QPM.

We have explored the potential of over-expressing QPM candidate genes in seeds of transgenic maize plants as a means to test their action as *o2* modifier genes and potentially, as a way to engineer vitreous endosperm modification in dominant low α-zein RNAi lines. In the case of PFP, over-expression led to unexpected growth inhibition in plants and was not pursued further. We investigated the biotechnological potential of another major QPM candidate gene, 27-kD γ-zein for not only modifying opaque kernel *o2* and α-zein RNA interference (RNAi) lines, but by protein remodeling, to directly biofortify the lysine and tryptophan content of the seed. So far, we have not been able to achieve high enough expression of engineered 27-kD γ-zeins to impact kernel lysine content.

Working on previous data implicating the 27-kD γ-zein as an *o2* modifier, we determined that the protein is essential for *o2* modification in QPM using a transgenic strategy in collaboration with Joachim Messing's group at Rutgers University. When an RNAi event was used to eliminate 27-kD γ-zein in QPM kernels, complete opaque reversion was achieved and we published this work in the *Proceedings of the National Academy of Sciences* in 2010

III) Impacts

- By identifying genes whose differential expression faithfully co-segregates with endosperm modification in QPM and recombinant inbred lines thereof, we have generated markers that can be used in future QPM introgressions such as Quality Protein Popcorn (see major research project 3 on page 14).
- Our work with PFP as a probable *o2* modifier, provided substantial new insight into the theory that non-ATP requiring glycolytic enzymes function in maintaining respiratory flux during energy crisis situations in the inner seed and other plant tissues that are subject to hypoxic conditions during stress.
- Confirming the essentiality and ubiquity of high 27-kD γ-zein protein across all QPM varieties, provides a reliable marker for endosperm modification during future breeding programs.

IV) Future directions

Over the next five years, 27-kD γ-zein and other marker genes will be invaluable markers as we progress with our breeding of Quality Protein Popcorn. We will continue to assess the success and possible utility of our engineered lysine-containing 27-kD γ-zein over-expression lines.

b) Studying endosperm maturation and protein quality using natural and RNAi induced opaque endosperm mutants

I) Background

Vitreous (hard) endosperm formation is an essential agronomic characteristic that underpins all of the uses of maize. We know zeins and zein protein body formation are intricately involved but there is still much to learn about this process and the role of non-zein accessory factors. We are characterizing opaque endosperm mutants since it is a good way to study protein body and vitreous endosperm formation. The mutants we use are naturally occurring or randomly induced through a forward genetics, transposon mutagenesis approach, or targeted through a reverse genetics, RNA interference approach.

II) Achievements

In 2007, before coming to UNL, I identified the molecular nature of the *Floury1* (*Fl1*) maize mutant, one of the oldest spontaneous maize mutants first described in 1912. This is a well cited paper in *The Plant Cell*. FL1 is a non-zein protein which resides in the membrane surrounding zein protein bodies and functions in efficient packaging of zeins during the storage assimilation phase in kernel expansion. As the first factor shown to have an accessory role in prolamin accumulation, the work highlights the value of further functional genomics using opaque endosperm mutants. At UNL, I have continued studying FL1 function in collaboration with Marisa Otegui at University of Wisconsin, Madison. In 2012, I co-authored a paper in *The Plant Cell* in which we described the unexpected storage of zeins and involvement of FL1 within storage vacuoles within the outer aleurone layer of endosperm, where zeins were previously thought not to accumulate.

Transposons are naturally occurring, mobile DNA segments that contribute to genome evolution, cause mutations and have been exploited for functional genomics in plant species including maize. I have been working on molecular characterization of several transposon induced opaque endosperm mutants that I screened from the *UniformMu* population at the University of Florida. I characterized the molecular basis of the *Mutator Tagged Opaque 140* (*mto140*) mutant. The *MTO140* gene encodes a member of the arogenate dehydrogenase family that functions in biosynthesis of the amino acid, tyrosine. Reverse genetic analysis using this gene sequence led to my identification of other members of this gene family in maize. I published this work as a corresponding author paper in 2010 in *Journal of Experimental Botany.*

Where gene sequences are known, as is the case for all zein genes, but mutants have not been isolated, the scientist must deploy reverse genetics strategies to create specific mutants in order to study the function of a particular gene. Loss of function, recessive mutants have not been described for zein genes, in part because of they are often highly duplicated and, in some cases, have a high degree of functional redundancy (multiple genes all contributing to the accumulation of a single protein type). For the γ-zein family, we investigated the extent of redundancy and non-redundancy for their role in protein body initiation and expansion using a series of γ-zein RNAi events in transgenic maize. We published this work in *Plant Physiology* in 2013.

III) Impacts

- Very high-level packaging of seed storage proteins is achieved through sequestration in both the endoplasmic reticulum in the endosperm and in protein storage vacuoles in other tissues (cotyledons and aleurone). We have advanced the understanding of this partitioning process through our study of FL1 and prolamin accumulation in both the starchy endosperm and aleurone tissues
- Characterization of *mto140* led us to identify and describe the maize arogenate dehydrogenase family, thus providing valuable basic information about amino acid biosynthesis in cereals
- γ-zein family members have significant functional non-redundancy, contrary to what was previously thought. The specific role of 27-kD γ-zein in protein body initiation helps to explain its role as an *o2* modifier.

IV) Future directions

One transposon generated opaque mutant that we identified and mapped turned out to be *opaque1* (*o1*) which was published by Rentao Song's group in *The Plant Cell* in 2012. Although the timing of our work meant we were unable to contribute to this paper, we have started collaborating with Rentao Song to study the functional relationship between FL1 and O1. O1 is a plant myosin (cytoskeleton) protein and FL1 has recently been shown to contain a myosin receptor domain. We are thus further investigating this likely functional interaction.

Our involvement in work on *mto-140* and tyrosine biosynthesis in maize is concluded.

For the zein RNAi lines, we will continue to explore the utility of a total α-zein RNAi event we generated as a dominant high-lysine template for testing the action of candidate *o2* modifier genes.

c) Functional genomics of maize seed development using gamma-radiation induced mutants and high-throughput mapping, DNA and RNA sequencing and proteomic profiling

I) Background

To build on previous broad mapping of QPM QTL regions, we conducted mutagenesis of a QPM line, in order to generate opaque revertant mutants. The rationale is that such mutants can be used to identify *o2* modifier genes and/or genome regions containing them. In addition this strategy generates novel mutations affecting kernel development in general, not specifically related to QPM. Both types of mutation are equally interesting and useful for advancing our understanding of kernel maturation.

We also created a separate mutagenized population, in the B73 maize reference background. The aim of this was to generate and characterize mutants specifically affected in kernel maturation and filling. The isogenic nature of the mutants with the B73 reference genome somewhat eases their molecular characterization compared with the QPM population which is not isogenic with B73.

II) Achievements

The QPM mutagenesis population was generated with limited field and manpower resources and thus consists of only 300 families. However, we identified more than 10 heritable, non-pleiotropic opaque revertants that we are in the process of characterizing. Two mutants have been advanced significantly more than the others to date.

The first mutant, line 107, has a deletion encompassing the 27-kD γ-zein gene, the first recessive null mutant described for this gene. We determined that the 27-kD γ-zein acts in a haploinsufficient manner to effect endosperm modification in QPM and published the full characterization of this mutant, as well as an introduction to the platform in *Plant Physiology* in 2014.

The second opaque revertant, line 198, has a very low level of residual α-zeins, compared with the QPM control. This could result from a physical deletion in a dominantly expressed α-zein gene cluster, or more likely, loss of a novel non-O2 regulatory factor. We have mapped this mutant and are currently comparing the map position with HiSeq-2500 genome sequence to identify the causative mutation.

The B73 mutagenesis population is much larger and contains 1793 families. Screening of approximately half of the M3 generation ears has so far led to the identification of more than 50, heritable opaque and small kernel mutants that have been crossed to Mo17, for which F2 mapping populations are being generated in summer 2014.

III) Impacts

- By combining a traditional method for inducing genetic variation with state-of-the-art DNA and RNA sequencing methods, we have demonstrated the feasibility of a new platform for seed functional genomics that can be applied to studying other traits in crop plants.
- We have demonstrated the utility of this method for creating new low-prolamin variants that have increased levels of limiting essential amino acids. Both line 107 and 198 have lysine and tryptophan levels significantly increased over wild type and even QPM controls.
- By focusing on the opaque and small kernel mutant classes in the B73 population, we have determined that there is considerable overlap between the mechanisms controlling formation of

vitreous endosperm and late stage endosperm - and thus grain - filling. By studying these mutants ourselves as well as making mutants, mapping data and sequencing data available to others in the maize community, we can substantially advance knowledge on key grain traits of grain texture and yield

IV) Future directions

Through our USDA AFRI foundational grant, we will continue charactering key mutants in both the QPM and B73 mutagenesis populations. Though we do not yet have federal funding for advancing the larger B73 population, I have secured ARD Bridge funding to map the first 50 B73 mutants, based on highly meritorious reviews from a 2013 NSF Plant Genome Research Proposal which has been revised and resubmitted in 2014. The goal for the B73 population is to fully characterize at least 30 small kernel and opaque mutants through mapping, DNA and RNA sequencing and full proteome, shotgun proteomics. All data and seed stocks will be made available to the maize community through a new web-based, searchable web site.

Major Research Area 2. Sorghum grain digestibility and protein quality

Sorghum, a drought-tolerant C4 crop and a close relative of maize, is grown in semi-arid, tropical or sub-tropical climate conditions where other cereals do not grow well. In Nebraska, sorghum has great potential for expansion as an animal feed since it can be grown on marginal lands. Like other cereals, high accumulation of prolamin storage proteins in the grain results in deficiency of lysine and tryptophan. In addition, the sorghum prolamins (kafirins) are packaged into highly indigestible, cross linked protein bodies. The amino acid and digestibility deficiencies can result in protein malnutrition, when sorghum is consumed as the primary protein source.

a) Characterized molecular identity of the High Digestibility High Lysine sorghum variant

I) Background

The High Digestibility High Lysine (*hdhl*) sorghum mutant was generated by chemical mutagenesis at Purdue University. The phenotype results in lower kafirin accumulation and severe reticulation of protein bodies that respectively result in increased lysine and dramatically improved protein availability through increased digestibility. The molecular basis of this mutant remained unknown for several decades, thus restricting its utility and impact.

II) Achievements

Through prolonged negotiation with Dr. Gebisa Ejeta and Dr. Bruce Hamaker at Purdue, I eventually secured release of the *hdhl* mutant to work on its molecular characterization. In collaboration with Dr. Joachim Messing at Rutgers University, we took a directed cloning approach and identified a dominantly acting amino acid substitution mutation in a kafirin signal peptide as the cause of the phenotypes. We published this work in *Nature Communications* in 2013

III) Impacts

- The *hdhl* variant has been shown to have market potential for improving human and livestock nutrition in the developing world
- Development of sorghum varieties with improved nutritional quality will enhance the appeal of sorghum as a marginal land crop in the Nebraska and other U.S. states.
- Knowing the molecular basis of the variant gives us a molecular marker for breeding
- Our paper presented pan-*Poaceae* comparison that identified critically conserved amino acids within prolamin signal peptides

IV) Future directions

Our role in the characterization of the *hdhl* sorghum variant is concluded. Though we are not currently breeding with *hdhl*, I would like to work with this variety especially for combining it with Nebraska sorghum varieties being developed by Dr. Dweikat and those they we generate in the mutagenesis population described below.

b) Creation of novel kafirin variants for improved digestibility and protein quality and sorghum functional genomics using gamma-radiation mutagenesis and high throughput mapping, DNA and RNA sequencing and proteomic profiling

I) Background

Based on the above negative nutritional and digestibility issues of sorghum grain, as well as its potential as a low input, marginal land crop, we have initiated a new mutagenesis population. This is funded as a seed project for two years by the ARD of IANR. Though the basic methodology is similar to that described for maize, sorghum presents several advantages over maize as described in 'impacts' below

II) Achievements

We propagated the first batch of M1 plants in the field and will screen M2 seed phenotypes in Fall 2014

III) Impacts (projected)

- Mutagenesis has been performed in a sorghum variety bred by Ismail Dweikat for Nebraska growing conditions, so new varieties will not require additional breeding for utilization in Nebraska
- A much larger M2 population is feasible because seed heads only have to be bagged requiring less labor to self-pollinate than maize
- The mutants are non-transgenic so that lines can be field tested and incorporated into breeding programs directly without the regulatory road blocks that restricts the transgenic varieties produced in the Africa Biofortified Sorghum program
- Low-kafirin variants simultaneously increase the protein quality and digestibility. The *hdhl* mutant is one example of the value of this although direct deletion has the potential for more complete kafirin abolition
- The sequenced sorghum genome is less than one third of the size of the maize and lacks the recent whole genome duplication in maize genome facilitating more cost-effective DNA-sequencing

IV) Future Directions

We plan to rapidly realize the utility of any lines we develop by collaborating with ICRASAT (International Crop Research Institute for the Semi-Arid Tropics), for which sorghum is one of their six mandate crops. Scientists at ICRASAT, Dr. Pinnamaneni Srinivasa Rao and Dr. Stefania Grando have expressed strong interest in this collaboration. ICRASAT's role will be to assist with introgression of selected mutants from our selected background into ICRASAT germplasms such as ICSV 93046, Macia and SPV 1411 which are adapted for growth in Asia and Africa in the follow up project. These introgressions can be performed both at UNL and at ICRASAT. ICRASAT will eventually conduct large scale phenotypic analysis for grain traits as well as field performance under local conditions.

Major research area 3. Breeding of Quality Protein Popcorn

I) Background

ConAgra foods are rebuilding their popcorn breeding program at UNL. I proposed to introgress Quality Protein Maize into elite popcorn varieties as one of several strategies to rejuvenate U.S. popcorn sales. Popcorn enjoyed nearly a constant increase in sales during the second half of the twentieth century but since this time, few technological advances have been introduced and sales of popcorn have waned. Revival of popcorn must include a new game changing development. *Quality Protein Popcorn* (QPP) may answer this call.

The funded work is a marker assisted breeding project which will select for the *o2*/*o2* mutant allele carrying the high-lysine trait and well as the QPM modifier genes that specify high 27-kD γ-zein and most importantly, vitreous endosperm. The critical component for success in this project is rapid recovery on the popcorn genome to enable acceptable popping quality. This will be done using new markers being developed by whole genome sequencing of multiple elite popcorn lines in collaboration with Aaron Lorenz.

II) Achievements

The project is in its infancy and two graduate students are beginning fall 2014 to work on popcorn marker development and the genetic and biochemical aspects of *o2* and QPM selection. So far, we have made F1 crosses between multiple different QPM inbreds and multiple ConAgra popcorn lines. In summer 2014, we generated BC1s for F1s from a prioritized group of four different QPMs. These QPM lines were selected based on agronomic properties, lysine content and vitreousness that I established during winter greenhouse tests.

III) Impacts (projected)

- **QPP is non-GMO.** Since QPM technology is based on a naturally occurring high-lysine *o2* variant and its modifiers, it is readily applicable for the development of non-GMO popcorn varieties, as mandated by current industry policy and consumer preference. While these policies and preferences may not be static, the approach described here represents an appropriate medium-term strategy. While I am fully supportive of the essentiality of GM traits in improvement of agronomic and quality traits of our crops, GM lines require massive monetary and time investments to bring to market.
- **QPP hybrids will maintain hybrid vigor.** By introgressing the *o2* gene and the modifier genes into multiple ConAgra popcorn lines, new hybrids can be produced which maintain the homozygous *o2* allele.
- **Increased pop volume.** Because it is the vitreous endosperm that contains starch capable of melting and rapidly expanding during popping, developing popcorn hybrids that contain elevated vitreous endosperm may facilitate increased pop volume. QPM varieties often have a more extensive proportion of vitreous endosperm compared with normal dent corn varieties and popcorn kernels also have more extensive vitreous endosperm than dent corn varieties. Therefore, development of QPM popcorn could drive higher vitreous endosperm and pop volume.
- **Enhanced nutrition.** Popcorn is a good source of dietary fiber, complex carbohydrates, protein, B vitamins and antioxidants such as zeaxanthin. Improving popcorn protein quality while maintaining optimum pop volumes and agronomic traits would broaden the appeal of popcorn as a healthful snack and could be promoted alongside the other nutritional attributes.

IV) Future directions

This is a new project, and the future directions have been described in sections I), II) and III) above.

Research Funding

Awarded (total **$1,154,020** of which $1,115,020 is awarded to and managed by Holding)

1. Gene discovery for sorghum response to nitrogen. United Sorghum Checkoff Foundation, project period, 03/20/2010 to 03/19/2011, **$39,000** (co-PI with Ismail Dweikat who managed funds).
2. Development of Quality Protein Popcorn as a non-GMO approach to enhanced nutritional quality, pop volume and flavor profile. ConAgra Foods, 9/1/2013 to 8/31/2018, **$532,035** (PI, Oscar Rodriguez and Devin Rose, co-PIs. PI currently assigned all funds)
3. A novel functional genomics platform for dissecting maize kernel maturation and protein quality. USDA AFRI, 11/1/2013 to 10-31-2016, **$412,985** (PI with Chi Zhang as co-PI. PI currently assigned all funds)
4. Engineering high protein quality and high digestibility sorghum through a novel non-GMO functional genomics platform. Externally reviewed, competitive, Center for Plant Science Innovation Seed Grant, 4/1/2014 to 3/31/2016, **$120,000** (PI, Chi Zhang and Ismail Dweikat, co-PIs. PI currently assigned all funds)
5. ARD bridge proposal to provide critical data for NSF PGRP proposal "An integrated functional genomics and proteomics platform for dissecting maize seed composition and size." Internal ARD award **$50,000** 6/1/2014 to 5/31/2015 (PI assigned all funds)

Pending

1. An integrated functional genomics and proteomics platform for dissecting maize seed composition and size. NSF Plant Genome Research Program 01/01/2015 to 12-31-2018, **$1,471,022** (PI, Chi Zhang, CoPI)

Peer reviewed research publications

Summary as of November 2014

	Total	Since 2009
Citations	546	365
h-index	12	11
i10- index	16	12

Work performed at UNL

***corresponding author**
(for UNL papers, Holding lab contributed 100% unless otherwise noted)

Gelli, M., Dou, Y., Konda, A.R., Zhang, C., **Holding, D.R.,** and Dweikat, I. (2014). Identification of differentially expressed genes between sorghum genotypes with contrasting nitrogen stress tolerance by genome-wide transcriptional profiling. BMC Genomics. 15: 179. (Designed experiments and advised graduate student on 70% of work, co-wrote paper. Dweikat provided genetic materials and funding). **Impact factor 4.40,**

*****Yuan, L., Dou, Y., Kianian, S., Zhang, C. and **Holding, D.R**. (2014) Deletion mutagenesis identifies a haploinsufficient role for gamma-zein in *opaque-2* endosperm modification. Plant Physiol. 164: 119-130. **Impact factor 7.1, Citations: 2**

***Holding, D.R.** (2014). Recent advances in the study of prolamin storage protein organization and function. Frontiers in Plant Science. Special issue: Advances in Seed Biology, 5:276. doi: 10.3389/fpls.2014.00276. Combined review and original research paper. **Impact factor 3.6**

Wu, Y., Yuan, L., Guo, X., **Holding, D.R.**, and Messing, J. (2013). Mutation Causing a Single Amino Acid Substitution Creates High Food Value Trait in Sorghum. Nature Communications, Volume 4, Published 8-16-2013. (performed 50% of work, co-wrote paper) **Impact factor 10.02, citations: 12**

*****Guo, X, Yuan, L, Chen, H, Sato, S.J., Clemente, T.E., and **Holding, D.R.** (2013). Non-redundant function of zeins and their correct stoichiometric ratio drive protein body formation in maize endosperm. Plant Physiol. **162**, 1359–1369 **Impact factor 7.1, citations: 5**

***Holding, D.R.**, and Messing, J. (2013) Evolution, structure and function of prolamin storage proteins. Invited, peer reviewed book chapter *in*: Seed Genomics, First edition, Wiley-Blackwell Publishing, Editor, Becraft, P. *pp138-158,* **citations: 4**

*****Guo X., Ronhovde K.J., Yuan L., Yao B., Soundararajan M.P., Elthon T.E., Zhang C., **Holding D.R. (2012)** Pyrophosphate dependent fructose-6-phosphate 1-phosphotransferase induction and attenuation of Hsp gene expression during endosperm modification in Quality Protein Maize. Plant Physiol. *158: 917-929* **Impact factor 7.1, citations: 4**

Holding, D.R., Hunter, B.G., Klingler, J.P., Wu, S., Guo, X., Gibbon, B.C., Wu, R., Schulze, J., Jung, R., and Larkins, B.A. (2011) Characterization of *opaque2* modifier QTLs and candidate genes in recombinant inbred lines derived from the K0326Y Quality Protein Maize inbred. Theoretical and Applied Genetics 122, 783-794 (performed 60% of work while at UNL, wrote paper) **Impact factor 4.01, citations 12**

Reyes, F.C., Chung, T., **Holding, D.**, Jung, R., Vierstra, R., and Otegui, M.S. (2011). Delivery of Prolamins to the Protein Storage Vacuole in Maize Aleurone Cells, The Plant Cell 23, 769-784. (20% of work at UNL) **Impact factor 10.13, citations: 50**

***Holding, D.R.**, Meeley, R.B., Hazebroek, J., Selinger, D, Jung, R. and Larkins, B.A. (2010) Identification and characterization of the maize arogenate dehydrogenase gene family, Journal of Experimental Botany 61: 3663-3673. **Impact factor 5.24, citations: 13**

Wu, R., **Holding, D.R.**, and Messing, J. (2010) Gamma zeins are essential for endosperm modification in Quality Protein Maize. Proceedings of the National Academy of Sciences, USA. 107: 12,810-12,815. (contributed research materials, evaluated data, co-wrote paper) **Impact factor 9.74, citations: 41**

Holding, D.R. and Larkins, B. A. (2009) Zein storage proteins, Chapter V. Invited, peer reviewed book chapter in: Molecular Biology and Physical Studies, in Molecular Genetic Approaches to Maize Improvement, (A.L. Kriz and B.A. Larkins eds.) pp 269-286. Springer-Verlag Publishers, Heidelberg, Germany. (wrote paper) **Citations: 23**

Before coming to UNL

Holding, D.R., Hunter, B.G., Chung, T., Gibbon, B.C., Ford, C.F., Bharti, A.K., Messing, J., Hamaker, B.R. and Larkins, B.A. (2008) Genetic Analysis of *opaque2* Modifier Loci in Quality Protein Maize. Theoretical and applied genetics 117: 157-170. **Impact factor 4.01, citations: 48**

Holding, D.R. and Larkins, B.A. (2008) Genetic Modification of Seed Storage Proteins. Invited peer reviewed chapter in: Advances in Plant Biochemistry and Molecular Biology (Lewis, N.G., Ed.-in-chief) Vol. 1, Bioengineering and Molecular Biology of Plant Pathways (Bohnert, H.J. and Nguyen, H.T, eds.), pp. 107-133. Elsevier Publishers, Oxford, UK. **Citations: 10**

Holding, D.R, Otegui, M.S., Li, B., Meeley, R.B., Hunter, B.G., Jung. R. and Larkins, B.A. (2007) The maize *Floury1* gene encodes a novel ER protein involved in zein protein-body formation. The Plant Cell 19:2569-2582. **Impact factor 10.13, citations: 55**

Settles, M., **Holding, D.**, Tan, B.C. Latshaw, S., Susuki, M, O'Brien, B., Fajardo, D., Wroclwaska, E., Lai, J., Hunter, C., Avigne, W., Peacock, S., Baier, J., Lonon, D., Messing, J., Hannah, L.C., Koch, K., Becraft, P., Larkins, B., and McCarty, D. (2007) Maize Sequence Indexed Knockouts using the UniformMu Transposon Tagging Population. (2007) BMC Genomics, 8:116. **Impact factor 4.40, citations: 43**

Holding, D.R. and Larkins, B.A. (2006) The development and importance of zein protein bodies in maize endosperm. Maydica 51 (2): 243-254. **Impact factor 0.37, citations: 19**

Lopez-Valenzuela, J.A., Gibbon, B.C., **Holding, D.R.**, and Larkins, B.A. (2004) Cytoskeletal proteins are coordinately increased in maize genotypes with high levels of eEF1A. Plant Physiology 135: 1784-1797. **Impact factor 7.1, citations: 16**

Holding, D.R. and Springer, P.S. (2002) The *VASCULAR PREPATTERN* enhancer trap marks early vascular development in Arabidopsis. Genesis 33 (4): 155-159 2002. **Impact factor 2.59, citations: 11**

Holding, D.R. and Springer, P.S. (2002) The Arabidopsis gene *PROLIFERA* is expressed at all stages of reproductive development and is required for cytokinesis. Planta, 214 373-382. **Impact factor 3.35 citations: 41**

Springer, P.S., **Holding. D.R**., Groover, A., Yordan, C. and Martienssen, R.A. (2000) The essential Mcm7 protein *PROLIFERA* is localized to the nucleus of dividing cells during the G1 phase and is required maternally for early Arabidopsis development. Development 127, 1815-1822. **Impact factor: 6.2, citations: 122**

***Holding, D.R.**, Springer, P.S. and Coomber, S.A. (2000) The chloroplast and leaf developmental mutant, *pale cress*, exhibits light-conditional severity and symptoms characteristic of its ABA deficiency. Annals of Botany. 86, 953-962. **Impact factor: 3.45 citations: 3**

Holding, D.R., McKenzie, R.J. and Coomber, S.A. (1994). Genetic and Structural analysis of five mutants with abnormal root morphology generated by the seed transformation method. Annals of Botany 74, 193-204. **Impact factor: 3.45 citations: 12**

Research presentations

Invited lectures presented at regional, national and international society meetings and/or other educational institutions

Holding, D. Nov 2014 invitation, "A novel functional genomics platform for dissecting maize kernel maturation and protein quality" Crop Science Society of America, Annual Meeting, Long Beach California.

Holding, D., June 2013 invitation, National University of Mexico UNAM, Mexico City, "Genetic, genomic, biochemical and cell biological insights into maize and sorghum endosperm texture and protein quality".

Holding, D., Feb 2012 invitation, BASF Plant Sciences, Durham, N. Carolina. "History, potential and molecular characterization of high-lysine, Quality Protein Maize"

Holding, D., Nov 2011 invitation, Nebraska Agribusiness Association, Kearney, NE., Extension Educators Meeting. "History, potential and molecular characterization of high-lysine, Quality Protein Maize"

Holding, D., Nov 2010 invitation, Animal Sciences, UNL, "New Insights into the characterization and development of Quality Protein Maize"

Holding, D., Apr. 2010 invitation, Food Sciences Department, Iowa State University, "The molecular genetics of kernel maturation and protein quality in maize"

Other evidence of national or international recognition

Ten poster presentations presented at international and national research meetings.

Additional scholarly service which demonstrates my value within my scientific field is my role as a journal reviewer. I have an international reputation in the field of seed storage proteins, protein quality, opaque kernel mutants and modified *opaque-2* (QPM). This expertise is highly valued since I am continuously asked to review papers within these areas. I review papers from multiple international journals (including Proceedings of the National Academy of Sciences, Plant Cell, Plant Physiology, Crop Science, etc) although I now try to limit this to two per month. I have been an invited reviewer for NSF proposals and USDA ARS research plans.

I will further strengthen my national and international reputation in maize genetics over the next five to seven years leading up to full promotion. This will be achieved in part through my work in generating maize kernel mutants for studying kernel maturation and quality traits. By pursuing novel ways to characterize these and make seed stocks, phenotypic data and *in silico* genome and transcriptome datasets available to others in the maize community, I will be contributing to forward and reverse genetics resources. Transposon mutant collections are the best available resources for forward and reverse genetics in maize but they are not saturated and sometimes mutant alleles are unstable. Thus, deletion mutagenesis, which creates mutations through physical gene loss, has attractive potential as a supplementary functional genomics resource. In the next year we will be able to fully appraise its potential through publication of complete genome sequences showing mutant maps for at least five mutants.

TEACHING (20%)

Teaching Philosophy and personal goals

Effective teaching of core concepts in plant science at the undergraduate level is imperative for the optimal progression of students pursuing agronomy and horticulture based careers. We must provide basic knowledge of fundamental science central to many subsequent advanced courses in a way that engages students and optimizes their learning potential while adapting to and utilizing novel teaching strategies. I believe that, as a department, we must optimally draw on the experience of our faculty to deliver basic and intermediate level plant biology. Young faculty with active research programs are best suited to energizing courses with the latest exciting scientific developments that relate to agronomic and horticultural aspects of plant growth. Although continual innovation in teaching methods is of paramount importance and is leading to radical new styles of delivery and learning outcomes in our department, we must continue to seek broad and wide participation, from our research focused faculty in basic plant science education.

In the past five years, I have fulfilled this role by teaching Plant Science (Agro 131). Agro 131 introduces Agronomy and Horticulture students as well as other CASNR and non-science majors to fundamental core aspects of plant science. These range from ecosystems and cycles, how plants make, use and store food, how plant diversity is reflected in metabolic differences between plants, how plants sense and signal short term changes in the environment to the introduction of core concepts of genetic control in all of the above areas. My passionate belief and overall goal is that Agro 131 or other similar introductory plant biology courses, if taught optimally and innovatively, will serve as the linchpin of plant science learning both for science and non-science majors.

Two broad sets of goals have influenced my Plant Science teaching:

1. I have sought to use my diverse skills in agronomy, plant anatomy, physiology, biochemistry, genetics and artistic design for optimal undergraduate teaching of core concepts of plant science. Between 2010 and 2013, I taught two of the four plant science units in the fall and spring. In fall 2013 and 2014, on the advice of the promotion and tenure committee, department head and mentors, I ceased teaching the smaller spring section. The units I teach relate to the fundamentals of how plants drive and control their growth. I am focused on core concepts to describe how all life depends on plants, and how these basic principles apply to crops of importance to Nebraska. My teaching aims to provide foundational principles in water properties and transpiration, light and dark reactions of photosynthesis, C3, C4 and CAM plants, respiration and integrating these concepts to discuss their effects on crop productivity. In controlling plant growth, I focus on plant responses to light, diagnosing and understanding the molecular basis of mineral nutrient requirements and deficiencies, crop domestication, control of nutrient accumulation during seed development, and introducing how plants use hormones to sense and adapt to their environment. I am primarily responsible for designing exams for the two units I teach.
2. I am driven to continually optimize my teaching style. I constantly strive to find the right balance between presentation of sufficient scientific information, and ensuring that the majority of students understand and retain the core principles. I use at least one interactive learning quiz per lecture and I continue to shift the balance towards proactive student participation. I used on-line pre-quizzing and feedback (ungraded) of core principles to highlight expected learning backed up by post-quizzing (graded) of materials for student focused appraisal of learning prior to examination. This has worked very well from both student and instructor perspectives.

Achievements

a) Undergraduate

In fall 2009, I assisted Don Lee in lectures and led recitation section. In spring 2010, I taught one out of the four course units (25% of classes) and from fall 2010 onwards, I taught the middle two of four units (50% of classes). Because the there are two weekly lectures and one recitation, my lecture contributions have been recorded as less than 50% of the course. This is because, apart from 2009, I have not taught directly, or received credit for, one of the 3-4 recitation classes offered in fall and spring. However, I have provided substantial material for the recitation classes in the form of problem sets and TA questions, and led TA meetings to guide the recitation classes. Division of credit for a course taught by multiple members, including teaching staff only involved in recitation teaching, is not straightforward. Indeed, my contribution to Agro 131 has been more substantial than is suggested by the percentages allocated to me. In addition to recitation materials above, I have developed materials including problem sets, in-class learning quizzes, exams, Adobe and Camtasia animations, revised presentations of previously covered material and introduction of entirely new elements in the curriculum. These materials are all extensively used by instructors in other sections and used in semesters in which I have not taught (spring 2013 and spring 2014). Although I gain much professional satisfaction and higher level recognition for these contributions, they are not reflected in my FTE percentages shown in Table 1. Furthermore, in 2013, on the advice of the promotion and tenure committee and the department chair, I switched to only teaching Agro 131 in the fall semester because I was judged to be over-teaching in relation to my research: teaching apportionment. This resulted in a calculated FTE of less than 20%. Again, this did not account for the contributions listed above.

Our experience in Agro 131 is that small groups of students perform better since they are afforded a greater level of personalized tuition. We are therefore moving more towards smaller sections covering the same core curriculum. Consequently, starting fall 2015, I will teach a new section in Beadle center, to ~30 students (*see Future Teaching Aspirations*).

Table 1. Calculated FTEs for 2009-2013

Year	Budgeted FTE	Calculated FTE
2009-2010	20%	32.62%
2010-2011	20%	30.14%
2011	20%	16.55%
2012	20%	24.34%
2013	20%	14.21%
		Av. 23.6

I have also been primarily responsible for writing and illustrating chapters relating to my course sections of our on-line text book:

*David Holding, Anne Streich, Leah Sandall and Donald Lee, "**Plant Science: The Biology of Plants Grown for a Purpose"** Great River Technologies*.

We continually update and optimize the eBook. For example, in 2014, we expanded the eBook problem pool and switched to using the eBook as 100% of the problem set grade, rather than having a separate problem set as part of the grade. This has resulted in greater completion of eBook readings by the students which was previously more difficult to enforce.

The evaluations I have received from students are shown in Table 2. With the exception of fall 2011 when I taught a small section in a Keim classroom, evaluations were administered by Don Lee, at the end of the course, several weeks after my teaching section had concluded. Sample size (n) reflects attendance during Dr. Lee's section of the course and does not coincide with the attendance of my lectures or the course enrolment which were higher. In summary, in the past five years, I have had a significant impact in the evolution of Plant Science 131 by introducing new coverage especially relating to the genetic, biochemical, biotechnological and agronomic aspects of seed crops of importance to Nebraska. I have implemented novel ways of appraise baseline knowledge of students and use the results to tailor my teaching of core concepts such as water balance, photosynthesis and respiration. This type of pre- and post-quizzing for the most fundamental elements has a significant impact on the preparedness of students for more in depth learning in courses such as Plant Physiology. During the Plant Physiology poster sessions, I have taken the opportunity to quiz former Plant Science students on their retention of core concepts I have previously introduced and been pleased with their progress from my basic teachings.

Table 2. Student evaluations for Agro 131 2010-2013

Category	Spring 2010 n= 57	Fall 2010 n= 124	Spring 2011 n= 44	Fall 2011 * n= 34	Spring 2012 n= 55	Fall 2012 n= 96	Fall 2013 n=68
General attitude	3.06	2.87	3.01	3.30	2.93	3.03	3.03
Method	2.76	2.47	2.67	2.86	2.60	2.59	2.44
Content	2.69	2.52	2.71	2.81	2.58	2.68	2.62
Interest	2.41	2.30	2.46	2.65	2.36	2.33	2.35
Instructor	3.03	3.00	3.08	3.36	2.99	3.02	2.78
Total	**2.80**	**2.65**	**2.80**	**3.02**	**2.71**	**2.74**	**2.65**

*** section in small classroom with extensive personal tuition**

Since 2010, each April I have taught one 90 minute guest lecture on 'Functional Genomics in Crops' to Animal Sciences 896 (Genome Analysis) taught by Daniel Ciobanu. This is an annually updated lecture which is always well received.

I have mentored two UCARE students. One of these, Alex Renaud, worked with me between fall 2009 and graduation in Spring 2011. His work included field work and field genetics studies. Alex is now graduating with his PhD in maize breeding from Purdue University and, in part due to his research experience with me and my mentorship, has secured a job at Monsanto in the Ag traits conversion team

b) Graduate

I have been the main advisor to two PhD graduate students Lingling Yuan and Kyla Morton (Ronhovde). Yuan successfully defended in July 2014 and graduated in August 2014 and secured a position at Li-Cor in Lincoln in August. Morton plans to defend in summer 2015. I was co-advisor to Malleswari Gelli, who graduated in December 2013 and is now working at Pioneer Hi-Bred in a senior position within the sorghum development program. A new PhD graduate student started in my lab in fall 2014. Under my supervision, Yuan and Morton received the Milton Mohr award in 2012 and 2013 respectively and Gelli received the Shear-Miles award in 2013. I have served on the committees of seven PhD graduate students in the past five years.

Future teaching aspirations

The table above illustrates the value of teaching small sections. In fall 2011, I taught a section with 36 students in a new classroom in Keim Hall. I was able to personally relate to all students and address them by name in class. This greatly facilitated interactive learning that is much more difficult in the Plant Sciences 102 room which is not a conducive venue for student-instructor interaction. Instead of two 50 minute lectures and a separate recitation, this consisted of a two 90 minute combined lecture and recitation format so that I also taught in the recitation sections. Although this entailed slightly more hands on teaching time for me, it paid dividends in terms of my satisfaction, and improved student evaluations and exam performance. The average exam grade was around 8-10 % higher than students taking the same exam in the large section.

Starting in fall 2015, I will begin teaching a small section in Beadle Center, similar to the 2011 course. I will teach the whole course (all four sections). It will be two 90 minute classes of combined lecture and recitation. This has several advantages for the students, the department and myself. For the students, it will offer greater potential for personal tuition in core concepts in plant science. With 25-30 students I will personally teach recitations along with a graduate student from my lab. With the facilities for growing plants in the Beadle Center greenhouses, we will be able to expand lab portion of the course. Students will conduct at least three experiments that they will write up and be graded on by myself. Offering the course in Beadle allows easier access by students living on city campus and for our department, this expands our realm giving us a greater presence and connection with Beadle center faculty and research. I will promote attendance at Biotech and Biochemistry seminars in Beadle and where appropriate, offer extra credit. We have found repeatedly, in evaluations, that students do not like having two different instructors, with different teaching styles, within the same semester. The advantages for myself are primarily that I take full ownership of the course and the learning outcomes. I can set my own exams based on what I teach rather than on an average of what is taught in several different sections. In addition it will significantly reduce the time I spend traveling between east campus and Beadle Center.

Local and broader impacts of my teaching, and alignment with department and CASNR missions

My teaching of plant science forges two main themes that provide impact at a local as well as a broader level. First, I cover basic core principles of plant growth and development so that students become fully cognizant of how all life is dependent on plants. Second, I relate these principles to agronomic and horticultural properties on plants grown for food, fuel, fiber and fun and wherever possible, to plants of importance to Nebraska. Students who are not native Nebraskans, or our growing number of on-line distance students, who may have taken the course to serve a basic interest in plants, thus gain a valuable applied perspective for how fundamental concepts relate to applied economic, or agronomically important traits. On the contrary, Nebraska students who are focused on a career in agriculture, may arrive with a preconceived notion that all they need is applied knowledge relating to farming, horticultural management or golf course management. I think most students are pleasantly surprised that they also gain a deep understanding how plants grow from basic as well as applied perspectives. Both types of students should ultimately know the significance basic aspects of plant growth and be able to answer questions like:

- Why corn is so productive in the Nebraska climate?
- What characteristics make seeds ideal for sexual plant reproduction and human and animal nutrition?
- Why is fruit stored in a low oxygen and low ethylene conditions?

We developed our on-line text book to serve the changing needs of our students. However, it is of course used by our distance students and has the potential to be used by students in other courses.

Teaching of introductory plant science is vital to the strength of our department and central to all aspects of its mission **"to advance the knowledge, theory, and application of plant and soils sciences and landscape design to improve the quality of life for citizens of Nebraska and the world"**. Though my

research touches on parts of this mission, my teaching contributes to the foundation on which all of our diverse courses build.

From the perspective of the missions College of Agriculture and Natural Resources (CASNR) teaching fundamental plant science is equally relevant and important. Although Agronomy and Horticulture is the largest CASNR and UNL department, many students from other CASNR departments as well as non-CASNR departments take Agro131 as is shown in Table 3. **"CASNR fosters a student-centered learning environment where diverse basic and applied natural, life, earth and social sciences are integrated into the context of a global society and environmental stewardship"**. Of all introductory course taken by students in other CASNR departments, Agro131 probably has the broadest appeal, providing applied knowledge of crop growth with sufficient contextual basic knowledge. Furthermore, we are striving to move towards student centric learning though our engaging recitations, and in-class group activities and finally, creating more course sections with smaller class sizes. With respect to the CASNR goal, **"to prepare students as leaders for a future in which demands on food, energy and water systems will challenge sustainability"**, students must attain a basic level understanding of how plants work in order to equip themselves for careers that address improving efficiency of crop growth. The research foci of many CASNR faculty are centered on improving crop performance under stressful conditions or limiting resources (input traits). It is imperative that students aiming for research careers either at UNL or elsewhere be well grounded in basic plant biology. The way I address sustainability in my research centers on improving seed nutritional quality (an output trait) as a means to improve feeding efficiency. I believe this will be a necessary part of meeting our future food requirements. In my teaching of seed biology, I take the opportunity to introduce elements of this research to the students.

Table 3. Enrollment in Argo/Hort 131 in fall 2013

College	Major	Enrol. Fall 13
CASNR (College of Agriculture and Natural Resources)	Agronomy	42
	Agribusiness	17
	Mechanized systems management	13
	Horticulture	8
	Turf grass and landscape management	5
	Environmental Science	3
	Agricultural economics	2
	Animal Science	2
	Fisheries and Wildlife	2
	PGA golf management	2
	Applied Science	1
	Environmental Restoration Science	1
	Grazing livestock systems	1
	Natural resources and environ. econ.	1
	Plant Biology	1
Arts + Sci	Sociology	1
Bus. Admin	Agribusiness	1
Ed.+ human sci.	Pre-elementary ed.	2
Undecided	Undeclared	2
	Visiting Student	1
	Total	**109**

SCHOLARLY SERVICE (2%)

Although I only have a 2% scholarly service appointment, I take this role very seriously. I believe that faculty should go above and beyond assigned duties. A department in which everyone did the bare minimum would quickly descend into a negative work environment, in which communication and cooperation was confined to individual research or teaching groups. In a department as large and diverse as ours, we must be proactive in our willingness to serve, not apathetic and assuming that someone else will pick up the baton. 'Paying it forward' by everyone will ultimately pay dividends for our collective and individual success.

As a member of the Center for Plant Science Innovation (PSI), like other members of our faculty based on separate campuses, I spend most of my working week at the Beadle Center physically separated from the day to day contact enjoyed by faculty based in Keim or Plant Sciences Hall. In addition to the many advantages, this also presents some challenges that I continuously strive to compensate for. On the advice of former and current mentors, and observing difficulties encountered by other PSI faculty in various departments, I take every opportunity to maximize interactions with Agronomy and Horticulture faculty. This includes attending and participating in all faculty and graduate student seminars, even when the topic seems disparate from my immediate interests. Indeed, such open mindedness often leads to novel insight and unexpected research interactions or student mentoring. Teaching on east campus has helped maintain this presence.

I have enjoyed my duties serving on departmental committees in the past five years. I served on the seminar committee from 2010 to 2012, during which I recruited faculty from outside Agronomy and Horticulture who work in the plant sciences, or agriculture-related non-plant sciences, to present their work. I served on the graduate committee from 2011 to 2014. I served on the search committee for the Plant Molecular Physiologist position in 2009 and was centrally involved in its successful outcome.

My PSI role involves additional scholarly service duties. I actively participate in the month to month discussions and planning that ensure the continued success of PSI. I often volunteer to host visitors and give guided tours of the core facilities in Beadle Center to diverse groups.

In 2012, I co-organized the Plant Sciences Retreat, held at the Lied Lodge in Nebraska City. For this I secured participation of key note, faculty, post-doc and student speakers, as well as chaired the sessions. I have been an active participant in the Biotechnology seminar series for which I have hosted three outside speakers.

I have contributed to a variety presentations for groups outside of UNL. In September 2011, I co-organized a "Sunday with a Scientist" day at the Nebraska State Museum. I gave a hands on presentation for all age groups that highlighted the role maize plays in our society from historical and current perspectives. I have often volunteered to host outside groups such as a group of 12^{th} grade science students from Lincoln East High School for which I gave lectures on nitrogen fixation in crops.

Other service of relevance to Nebraska is that I have written and illustrated sections of an extension publication for Master Gardeners with Anne Streich. I wrote and illustrated a gardener's perspective of water balance, photosynthesis, respiration and sugar translocation in its entirety and contributed to the section on plant mineral nutrition. These were peer reviewed internal to UNL.

During an invited trip to the National Autonomous University in Mexico City (UNAM) in June 2013 in which I presented my research, I was also invited to give a talk about study and research opportunities at UNL for grad students and post docs.

Additional scholarly service which demonstrates my value within my scientific field is my role as a journal reviewer. I review papers from multiple international journals (including Proceedings of the National Academy of Sciences, Plant Cell, Plant Physiology, Crop Science) although I now try to limit this to two per month. I have been an invited reviewer for NSF proposals and USDA ARS research plans.