ACCESSION NO: 1015621 **SUBFILE:** CRIS
PROJ NO: WNP00008 **AGENCY:** NIFA WN.P
PROJ TYPE: HATCH **PROJ STATUS:** NEW
START: 20 FEB 2018 **TERM:** 30 SEP 2022

INVESTIGATOR: Kirchhoff, H.; Lange, BE, MA.; Peters, JO, .; Sanguinet, KA, .; Smertenko, AN, .; Tanaka, KI, .

PERFORMING INSTITUTION:
WASHINGTON STATE UNIVERSITY
240 FRENCH ADMINISTRATION BLDG
PULLMAN, WASHINGTON 99164-0001

MOLECULAR PLANT SCIENCES (MPS): PLANT PRODUCTIVITY IN A DYNAMIC ENVIRONMENT

NON-TECHNICAL SUMMARY: This new molecular plant science (MPS) Hatch umbrella project will provide advanced fundamental knowledge in different areas of plant biology that will help to improve crop plants under increasingly challenging environmental conditions. Gaining this basic scientific knowledge will pave the way not only for improving existing but also for identifying new strategies to bioengineer crops. Specifically, detailed insights into primary and secondary metabolisms addressed in the focus area Products/Synthesis would improve production of economically-valuable chemicals in plants. Furthermore, the project will study interactions between roots and their biotic (microbiome) and abiotic components of soil. Understanding these interactions would enable breeding crops for profitable farming on marginal lands. Finally, understanding how plants sense their environment in general and stress in particular addressed in focus area Defense/Signaling will provide critical information for protecting plants from damaging impacts of anticipated global climate changes. In this way, the Hatch umbrella consortium will help solve pressing societal problems related to agriculture.

OBJECTIVES: The overarching goal of this Molecular Plant Science (MPS) umbrella project is to advance our understanding of the molecular basis of plant and crop productivity and to describe mechanisms how plants survive and thrive in challenging, changing, and often unpredictable environments. This goal addresses five of the six USDA-NIFA challenge areas (Food Security, Climate Variability and Change, Water, Bioenergy, and Food Safety). Although this MPS umbrella consortium focuses on basic aspects of plant biochemistry, physiology, and molecular biology, the scientific knowledge gained here lays an urgently needed foundation for groundbreaking advances in crop productivity and quality as well as crop environmental robustness. The overall goal of this project will be accomplished by the following objectives: 1. Characterization of potential factors that control architectural dynamics in photosynthetic membranes. 2. Identify the rate-limiting processes of starch synthesis and accumulation. 3. Engineer desaturases from a cyanobacterium and a nematode to efficiently reduce saturates during the synthesis of triacylglycerol, the principal component of seed oils. 4. Cloning, recombinant expression, and biochemical and functional characterization of enzymes participating in flavin biosynthesis in plants and bacteria. 5. Identify

and/or introduce biochemical pathways to valuable specialty and commodity chemicals (including medicinals) in plants and algae and identify regulatory point controls affecting their amounts produced from photosynthetically produced carbon capture. 6. Characterize important metabolic pathways (genetic impact/control) in important culinary herbs, aromatic plants, and berries that are involved in bioactive compound production with a particular focus on the pathways that produce the curcuminoids, salvinorins, and flavones and identification of the factors that regulate those pathways. 7. Develop spectral databases to support the rapid identification of isoprenoid natural products from complex mixtures. 8. Elucidation of encoding and decoding mechanisms of intracellular Ca2+ signature into various physiological responses through calmodulin and other Ca2+-binding proteins in plants. 9. In-depth understanding of a role for danger signals during abiotic and biotic stresses. 10. Identification of how genes and pathways regulate plant growth and development and then use breeding, transgenic, and genome editing approaches to change gene activity in crops. 11. Determine signaling processes between plant cells and pathogens using the pea endocarp tissue model and the potato excised stem model. 12. Determine genetic mechanisms responsible for regulation of peroxisome abundance in wheat and importance of peroxisomes for drought tolerance. 13. To understand the mode of action of the C-Ph1 gene to answer fundamental questions related to the process of chromosome pairing and homology search. 14. Molecular, genetic, and biochemical characterization of the BUZZ kinase in B. distachyon and A. thaliana. 15. To provide a fundamental framework to improve productive symbiosis and better nitrogen management of associated crops. 16. Provide a fundamental understanding of the regulation of ammonia synthesis in free-living nitrogen fixing organisms using A. vinelandii as a model system. 17. To study root-knot nematode secretions to identify and characterize root-knot nematode parasitism genes.

APPROACH: Due to the diverse research areas defined in the MPS Hatch umbrella a wide set of different techniques and methods will be employed. The following succinctly summarizes these techniques and methods. 1. For studying ultrastructural dynamics in photosynthetic thylakoid membranes state-of-the-art electron microscopy will be used complemented by a battery of biochemical, biophysical, and functional characterizations and computer modeling. 2. RNAseq and metabolomic approaches as well as a variety of biochemical and molecular biological approaches (proteomic analysis, immunoprecipitates, yeast two-hybrid and BiFC, in situ RT-PCR, knock-out mutations identified by TILLING, RNAseq) will be employed to study changes in gene expression and metabolites in transgenic rice lines and the identification of RNA binding proteins of the cortical endoplasmic reticulum in developing rice endosperm. 3. Transgenic approaches will be employed to supply of reductant to fatty acid desaturases that will boost the production of high-oleic oils. Sophisticated transformation vectors will be used to produce multi-transgenic soybean and canola lines for optimized oil compositions. 4. Cloning, recombinant expression, and biochemical characterization of recombinant enzymes will be performed to modify proteins involved in flavin biosynthesis. 5. Multi-omics (transcriptomics, proteomics and metabolomics) approaches at the whole plant, tissue, cell type, or subcellular level, gene cloning (typically the cDNA), recombinant protein expression, together with conventional biochemical and chemical pathway/intermediate identification are employed to identify how to maximize accumulation of valuable plant secondary and bioactive metabolites. 6. Online resources and published literature will be surveyed to generate novel spectral records for integration into the Spektraris family databases. 7. Molecular, biochemical and transcriptomic analyses on Arabidopsis mutants will be performed to show the impact of deleted domains on subcellular localization of the modified AtSR1/CAMTA3 proteins, the effect of calmodulin-binding to AtSR1/CAMTA3, and to determine their ability to regulate EDS1 expression. Whole-Genome Tilling will be used to detect the direct downstream targets of AtSR1/CAMTA3. 8. To test how the activated P2K receptor contributes to trigger defense responses functional analysis of the P2K receptor in defense responses against pathogens and insects will be performed complemented by pathogen infection assays. Co-immunoprecipitation

experiments are planned to identify additional downstream components that comprise the P2K receptor complex, using LC-MS/MS confirmed by Bi-FC and yeast two-hybrid assay. Furthermore, for studying extracellular ATP signaling pathway, RNA-seq and phosphoproteomics analysis will be employed. New danger signals in pathogen-infected hairy root of potato plants will be identified by NMR or mass spectrometry. 9. Reverse genetic screens, gene editing, and mutagenized TILLING populations in Arabidopsis, camelina, canola, Brachypodium and rice will be employed to identify the roles of genes associated with BR-inactivation, seedling and adult development. Reverse genetics will include the use of transgenic plants as well as CRISPR/Cas9-based gene editing. By using a combination of forward genetics, molecular genetics, genomics, and traditional breeding new varieties of grasses (Kentucky bluegrass, the ancient grain teff, western wheatgrass, prairie june grass, tufted hairgrass, bread wheat) that can be used for agricultural and horticultural purposes will be identified. 10. The potato excised stem assay and pea endocarp tissue will be used to analyze the critical initial development and defense gene activation. 11. Peroxisome abundance in Kronos TILLING population will be determined in tissue extracts. The effect of peroxisome abundance on drought tolerance will be assessed in greenhouse conditions. Fitness will be measured as a grain number, total weight of grains per spike, and number of spikes. Functional conservation of identified genes will be examined the using reverse genetics in Arabidopsis and Brachypodium. 12. Methods for targeted transfer of genes from wild relatives into crops without the 'linkage drag' of unwanted chromatin will be developed. This will be done by transient silencing of the C-Ph1 gene by virus induced gene silencing or RNAi. 13. Molecular, genetic, physiological, biochemical, and -omics-level approaches will be used for comparative studies of the BUZZ kinase. To determine the target proteins of the putative BUZZ kinase, we will use comparative phosphoproteomics to compare wild-type BUZZ and the buzz mutant complemented by co-immunoprecipitation (co-IP) assays. EMS mutagenized lines will be screened to identify buzz suppressor loci using high through put phenomics. 14. Bacterial genetics will be used to probe the symbiotic state of nitrogen and carbon metabolism and to monitor the activity of bacterial genes during symbiosis. A unique nitrogen-fixing but ineffective bacterial mutant will be employed that is affected in a stress response regulatory gene and involves a plant defense mechanism. 15. Gene replacement strategies will be employed to place defined modification into the two-component regulatory gene system nifL and nifA in A. vinelandii. Diazotrophic growth phenotype, patterns of gene expression, and ammonia production in mutant strains with defined deletions in regions of nifL and nifA will be used to delineate regulatory control factors. 16. Root-knot nematode effectors will be used as molecular probes in yeast two hybrid assays to find their potato interaction partners. By obtaining information about nematode secretions and the plant proteins they target in the host we will identify plant processes that will be the focus for potato resistance engineering.

KEYWORDS: Climate Change; Food Safety; Plant Biology; Bioenergy; Economically-valuable plant chemicals; Plant stress biology; Molecular Plant Science