How will elevated [CO_2] alter osmotic adjustment of *Arabidopsis thaliana* ecotypes under drought? *Keywords: Arabidopsis thaliana, elevated [CO_2], drought, osmotic adjustment*

Objective I propose to undertake an integrated investigation of physiology, gene expression, and biochemistry of *Arabidopsis thaliana* grown under elevated [CO_2] and reduced soil moisture. Specifically, I will examine the impacts of these climate change factors on the osmoregulation capabilities of six ecotypes of *A. thaliana* and the plasticity of this trait under elevated [CO_2]. This project will address issues at the intersection of ecology, physiology and molecular biology and will utilize skills I have already developed in each of these disciplines.

Introduction Increases in global atmospheric carbon dioxide concentration ([CO_2]) from the current values of 380 ppm to 550 ppm by 2050, coupled with future decreases in local water availability will have dramatic impacts on plant performance[1]. Elevated [CO_2] generally stimulates photosynthesis and growth in C_3 species[2]. In addition, elevated [CO_2] reduces stomatal conductance and water use, causing increased water use efficiency[2]. More efficient water usage is beneficial in ecosystems where water is limiting net primary production. Water deficit in plants also reduces stomatal conductance, causing higher water use efficiency, and in extreme cases, causing mortality[3]. However, the effects of elevated [CO_2] and drought on plants are non-additive[4]. If the soil dries, leading to a lower soil water potential, then the plant must lower its water potential. This response is called osmotic adjustment and is controlled primarily at the cellular level[5] through the accumulation of osmotically active metabolites (osmolytes). Metabolites which play a role in osmotic adjustment include: proline, glycine, serine, sucrose, glucose and fructose[6]. The biosynthetic pathways of these osmolytes have been, at least, partially elucidated[6]. Osmotic adjustment has been demonstrated to be key factor determining the degree of plant drought tolerance[3]. However, we do not know the extent to which osmotic adjustment will be enhanced by elevated [CO_2] to improve plant drought tolerance, how this enhancement varies with respect to specific osmolytes, or how it varies across ecotypes. My undergraduate research has shown that even in non-drought conditions soybean grown under elevated [CO_2] had greater water potential than soybeans grown under ambient [CO_2], which correlates directly with increased concentrations of osmolytes under elevated [CO_2][7]. By utilizing the simplicity of an *A. thaliana* system, I can better investigate the details of these mechanisms. The suite of molecular and genetic tools available for this species makes it the best choice with which to detect and quantify the mechanisms underlying intra-specific variation and plasticity of this trait.

Hypotheses

1) Under low water availability, ecotypes from dry locations will more effectively alter transcription of the signaling and metabolic components of osmoregulation. This will cause osmolyte accumulation, in turn increasing leaf osmotic potential, and thereby sustaining leaf water content and physiological activity.
2) Growth at elevated [CO_2] will increase photosynthesis and transcript abundance of metabolic enzymes at key steps in carbon and nitrogen metabolism allowing greater allocation of resources to osmotically active metabolites.
3) Transgenic knockouts of key osmolyte biosynthetic enzymes will display metabolic flexibility allowing them to use other osmolytes for osmotic adjustment, and this plasticity will be enhanced under elevated [CO_2].

Experiment 1; assessing hypotheses 1 and 2: *A. thaliana* (Col, aa-0, ag-0, cvi-0, ka-0 and wl-0)[8] ecotypes, varying in water availability of source location, will be grown in environmental growth chambers allowing temperature, light and [CO_2] control. Target [CO_2] treatments, achieved by a computer-monitored controller will be 380 ppm(ambient) and 550 ppm(elevated).

Drought will be induced in both CO_2 treatments by withholding water ten days prior to measurements[9]. I will assess the effect of drought and elevated [CO_2] on the transcript abundances for enzymes involved in the synthesis of osmotically active metabolites. This will be coupled with photosynthetic gas exchange to determine the factors limiting carbon uptake. To validate the impact of altered gene expression on osmotic adjustment, I will measure the pool size of the metabolites of interest, total water potential, osmotic potential, turgor pressure, specific leaf area, and carbon to nitrogen ratios of foliar tissue. Using full-sibling families of each ecotype grown under similar and contrasting conditions will allow me to determine to what degree this trait is genetically regulated.

Experiment 2; assessing hypothesis 3: Growth of *A. thaliana* knockouts of key osmolyte biosynthetic enzymes (determined from experiment 1) will enable the estimation of the importance of each osmolyte to general metabolism, osmotic adjustment plasticity, and which osmolyte(s) will benefit most from elevated [CO_2]. For example, I will use publicly available lines that do not produce Pyrroline-5-carboxylate reductase, which is essential in the production of Proline. This would allow me to compare osmotic capabilities without a major osmolyte.

Methods Gas exchange and chlorophyll fluorescence will be measured on leaves using a LiCOR 6400 open gas-exchange system[10]. Total relative water content, leaf water potential, osmotic potential and leaf turgor will be measured by the dew point psychrometer method using a microvoltmeter[10]. Tissue will be collected and transcript abundance of key enzymes of osmolyte synthesis characterized using RT-PCR[11]. Significant changes in transcript abundance under drought and elevated [CO_2] will be visualized using a custom display within the Mapman program[12]. Osmotically active amino acids, sugars and sugar alcohols will be assessed in an Agilent gas chromatograph and MS system. Specific leaf area and carbon to nitrogen ratios will be assessed from leaf punches in a COSTECH CHN analyzer[10].

Broader Impact This project will advance basic and applied plant biological research. We have already established a good understanding of the average responses of C_3 plants to elevated [CO_2] and drought, but have a limited mechanistic understanding of the molecular or genetic variation in responses. By using multiple scales including populations, individuals, leaves, cells, and molecular data, this project provides an integrative assessment that is essential for interpreting plant responses to these multifactor changes[4]. This information will be useful in identifying targets to select for in crops which will allow greater drought tolerance under elevated [CO_2]. It could also be included into more detailed versions of current climate change vegetation models[13]. I will incorporate my findings into the lessons of the outreach program I described in my personal statement. Midwest Alliance STEM students will continue to actively participate in hypothesis testing, data collection, and analysis within the contexts of this proposal through independent research projects.

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