LINDSEY, RAYMOND S.[1] and J. MITCHELL McGRATH[2]*, [1] Senior, Undergraduate Student, Crop and Soil Sciences, Michigan State University and [2]USDA-ARS, SBRU, 494 PSSB, Michigan State University, East Lansing, MI 48824-1325. **Examining salt stress for improvement of seedling vigor.**

ABSTRACT

Emergence and stand establishment through the first 10 weeks after planting continue to be primary concerns of sugar beet growers worldwide. Our goal is to understand the genes and genetics of seedling vigor, with vigor defined here as the ability of seedlings to tolerate adverse environments. Ongoing work shows an apparent induction of vigor via hydrogen peroxide that results in mobilization of stored lipid reserves for heterotrophic growth in good emergers, and that exogenous hydrogen peroxide can rescue the adverse effects of NaCl stress on germination percentages. We used this method to screen a series of adapted and unadapted germplasm to identify for tolerance to NaCl during germination, and selected and enhanced a number of germplasms that perform reasonably well under ion toxicity stress, notably progeny derived from Ames3051. Characterization of Ames 3051 progeny showed that germination was retarded in 75 mM or 150 mM NaCl relative to water or hydrogen peroxide, but final germination was not significantly affected. Candidate genes were identified from the literature that were shown to be involved in response(s) to salt stress, and their cognate genes were identified from sugar beet nucleotide sequence collections. These will have been used to examine differential gene expression during salt stress germination. Accumulation of small molecule metabolites (proline, glycine betaine) quantified via mass spectroscopy will help to characterize the mechanisms of salt stress tolerance in sugar beet.

Sugar beet, a salt tolerant crop at adulthood, is vulnerable to salinity during germination. The goal of this research was to examine the response of sugar beet to saline conditions, ultimately to provide germplasm that could have an agronomic and remediation uses in saline soils around the world. Accessions and treatments were selected from previous work (McGrath et al., Acta Horticulturae 782: 35-48, 2008). This was completed through examining the germination of sugar beet in saline treatments in the laboratory. It is thought that there may be multiple mechanisms of defense for the sugarbeet when exposed to saline conditions. Our current work has sought to examine gene expression and metabolite accumulation as it applies to seedling tolerance of saline conditions. Results from this research also contribute to the larger goal of finding innovative methods to assist in the process of land reclamation. The ability of the sugar beet to survive saline conditions makes this crop a potential candidate to aid in land reclamation in relation to high soil salinity, given that germination rates can be increased.

Initially, germination on plates and in solution was compared. Results suggest that the agar plate presented a slightly more stringent selection pressure (Table 1), since germination in 200 mM NaCl solution was greater than in 200 mM NaCl on agar plates, and other treatments were generally not statistically different (Figure 1). Salt concentrations above 200 mM NaCl were generally inhibitory to germination. Viability (as measured by 0.3% H_2O_2) and vigor (as measured by H_2O) were generally good but hydrogen peroxide appeared to have a detrimental effect on EL55 germination.

New sources of salt tolerant germplasm were identified in a preliminary set of solution experiments. Seed for selections for this experiment were increased in bulk and harvested by seed parent family. Three of these families as well as two controls (EL-A015035 = USH20 and EL-A012255 = ACH185) were tested in the agar plate environment, using three NaCl concentrations (Table 1). EL-A022423 from PI 232889 (‘Eszterhazai Voros’ from Hungary = IDBBNR 5405) and a specific high germination selection from Ames 3051 (EL-A022799)

showed excellent germination at higher salt concentrations, while EL-A022420 (from PI 355963) was intermediate (Table 1).

Table 1: Germination of sugar beet seed on plates supplemented with various salt concentrations.

ID	0.3% H_2O_2	sd	50 mM NaCl	sd	100 mM NaCl	sd	150 mM NaCl	sd	H_2O	sd
EL-A022423	19.7	0.6	19.3	0.6	19.7	0.6	16.7	1.2	20.0	0.0
EL-A022799	19.3	0.6	18.7	1.2	18.0	1.0	16.3	0.6	20.0	0.0
EL-A015035	18.0	1.0	15.7	1.7	13.0	4.6	13.0	2.1	16.7	1.6
EL-A022420	20.0	0.0	19.0	4.0	15.3	3.5	10.3	1.0	20.0	0.0
EL-A012255	16.7	1.2	14.0	2.6	15.7	2.5	9.0	2.6	16.7	2.1

To examine gene expression under saline conditions, a pool of candidate genes has been assembled. These genes are influential in many different aspects of cellular processes; those being examined in this study are metabolite pathway and antiporter genes, which are used in the exclusion of excess sodium from entering the cell, assisting in maintaining osmotic balance.

Table 2: Candidate genes and their role in salt tolerance within the cell.

NCBI Accession	Primer Sequence (f,r)	Function Of	Role in Cell
BQ589958	TTGTTTTTCCCCTTATCTTTGTTA, ATTTCCCCTTAGCTGTGTATGC	ATNHX1	Na^+/H^+ Antiporter
BQ591646	GGGGGAGTVVTTGATGGAATGATG, TAAAACCGAGCCACAACACTGATG	SOS1	Na^+/H^+ Antiporter
BQ587584	AGGGCGTTCTTAAAGTCTCTGATT, GTGATACGCGTTGAAGGATTAGG	SOS2	Na^+/H^+ Antiporter
BQ583653	TATGGGCTGCTGCTATTCAAAAA, ATACAACGCCTCCACTTCACTCTC	SOS3	Na^+/H^+ Antiporter
CF543338	GGGCGGTTGACCATTACATTAGAC, GCACCAAAACCACGGCTTCC	betA	beta-Amylase production
BI073169	GGTGATATTCCGGCGGGCAGTTC, GGGTGACGGGAGCTTTTTGTTTAG	BADH mRNA synthesis	Glycine -Betaine Synthesis Pathway
BF011111	TGTTTGATGCTGGCCTTCTGTCTT, TTGCCGGAGTTGTTGTGTCTTTTG	GSR	glutathione reductase
BQ582868	GACCCCGAAATTCCACCTGA, GAGCGATCCATCTAAGCCATACAC	choline mooxygenase synthesis	Choline Synthesis Pathway
BQ588320	ATAAAAATGGCGATCAAGACGACT, CATCAGAAAAACGGCCAAGAAGG	ENCODES A CHOLINE SYNTHASE	Choline Synthesis Pathway
BQ592298	TGAGGCGCAAAAGGGTGAA, AGAGGAAGGTGCGTATGTGAAATC	SOD H+ Antiporter	Na^+/H^+ Antiporter
BQ588320	ATAAAAATGGCGATCAAGACGACT, CATCAGAAAAACGGCCAAGAAGG	choline kinase	Phosphocholine Synthesis
CF543585	CCACGCGTCCGACATGCTACACTG, CGACCTGCAAAACCATACCAAGAA	HKT1 mRNA Synthesis	Controls Na+ entry into roots
BQ594545	ACCCTTTTCGTCATCACCTACTTA, AACCGCCTTCAGCAGCAATCT	BAS1	Blocks α-amylase production

In other plant species, differentiation in accumulation of key metabolites has been shown to largely affect the growth and health of the plant under saline conditions. By analyzing the levels of these metabolites, it may be possible to predict the ability of an accession to tolerate

saline conditions. Metabolite accumulation within the plant cell when exposed to different saline concentrations was examined. While some of the candidate genes already discussed are involved in metabolite accumulation, a separate study is investigating the levels of specific metabolites that have been shown in other plant species to have a beneficial impact on the plant's ability to tolerate salt or osmotic stresses. It has been shown that under saline conditions proline accumulation may increase, proline betaine is of importance in alfalfa under saline conditions and in many studies the importance of glycine betaine has been shown. Table 3 shows the metabolites that were analyzed in the samples using the MSU Metabolomics Facility and their Quattro Micro API to conduct LC/MS/MS analysis. Samples were prepared by germinating seeds on filter paper which had been soaked in either concentrations of H2O, 75 mM NaCl, or 150 mM NaCl. Seedlings were then freeze-dried and samples were prepared according to the Metabolomics Facility protocol.

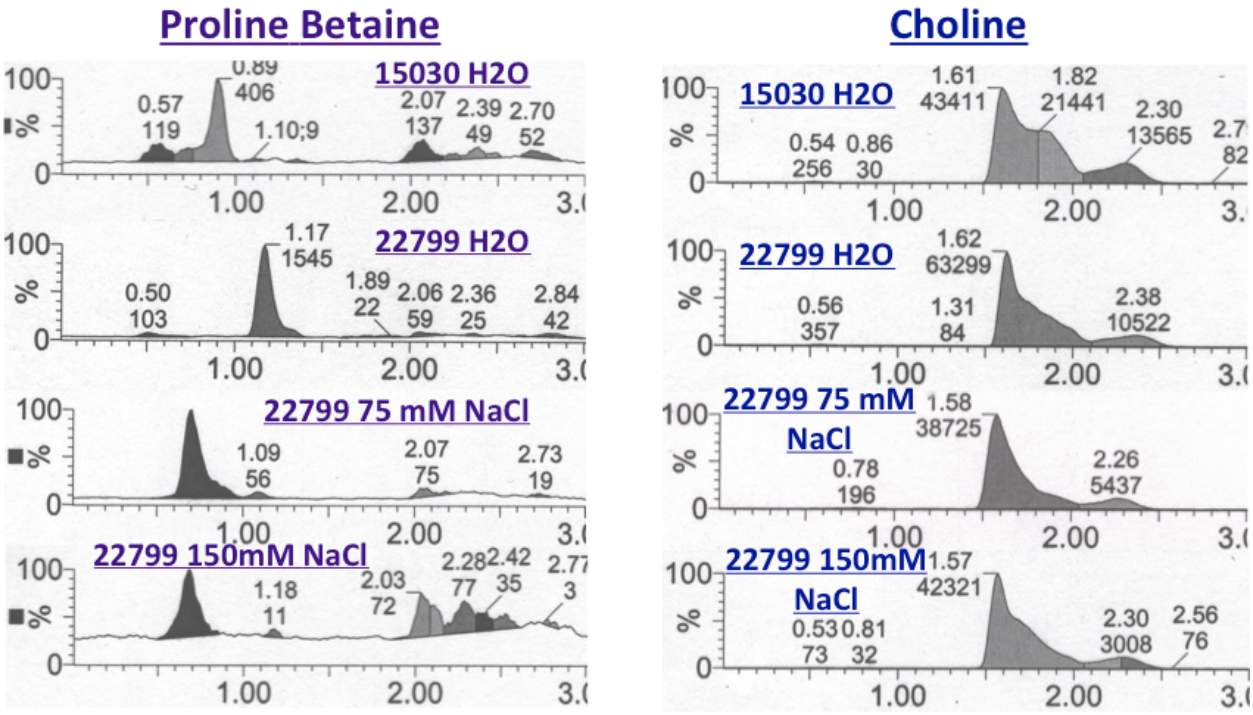
Table 3: Metabolites examined via LC/MS/MS.

Metabolite	Transition High	Transition Low	CV	CE
Glycine Betaine	118.1	58.78	34	16
Betaine Aldehyde	102	59.9	46	16
Choline	104.03	59.8	46	16
Proline	115.96	69.9	34	16
Sarcosine	90	44	25	20
Azetidine-2-Carboxylic Acid	102	56	25	20
Proline Betaine	144	84	34	16

Preliminary results from the metabolite studies have shown that there is a difference in the accumulation of these metabolites within varieties and treatments tested. Measurements of the area under the peaks allow for relative comparisons to be made comparing the differing treatments applied. As best exemplified by the glycine betaine (Figure 1), it is observed that there is a relative decrease in accumulation of glycine betaine that follows approximately a logarithmic decline as salinity increases. Choline was also seen to follow a similar pattern (Figure 1), except that past the rate of 75 mM NaCl accumulation of choline within the plant material ceased to decline. Comparing the variety EL-A015030 to the more salt tolerant EL-A022799 accession, it is observed that at the H_2O baseline EL-A022799 has a greater accumulation of these measured metabolites. It may be because of this that EL-A022799 is more salt tolerant; beginning with a higher accumulation of these metabolites that assist in the salt tolerance of a plant, it may maintain a higher level of these metabolites at all levels of salt treatment when compared to less salt tolerant accessions.

Examining the time at which each metabolite peaks also offers another possible way to screen for salt tolerance among sugar beet accessions. Taking glycine betaine as an example, it can be seen from Figure 1 that in the more salt tolerant EL-A022799 lines the peak was reached at a higher point in the H_2O control than for either the 75mM or 150 mM NaCl treatments. Comparing this to the H_2O treatment for the EL-A015035 line, the peak for this variety did not show a similar delay. More research is needed to conclude with any certainty but it may be a possibility that these differences in peak times may be a useful screening tool to identify those accessions which are more salt tolerant.

Figure 1: Accumulation of two salt stress metabolites during germination.



For glycine betaine levels in collected samples, EL-A022799 showed a decrease in area under the peak: 236,274 for H_2O Treatment, 34,047 for 75 mM NaCl, 2032 for 150 mM NaCl. Choline levels in EL-A022799 showed a significant decrease in area under the peak between H_2O (63,299) and 75mM NaCl (38,725) treatments. Proline betaine levels in EL-A022799 showed a decrease in area under the peak: 1545 for H_2O treatment; 56 for 75mM NaCl, and 11 for 150 mM NaCl. Interestingly, these values were roughly the same in treatments where the germination begins to be inhibited (i.e. 150 mM versus the control values of salt-sensitive EL-A015030 germinated in the absence of salt).

Future work will consist of further examining these metabolites in different sugarbeet accessions. The relation between these metabolite accumulations and candidate gene expressions may shed light on the role that these candidate genes play in increasing salt tolerance in sugarbeet.