GREENHOUSE AND FIELD SCREENING FOR SALINITY TOLERANCE

Peter Reisen[1], John Dodd[2], Yun Kang, Michael Udvardi[3], Paul Tracy[4], Amaia Seminario[5], Julie Ho[6], Mark McCaslin[7].

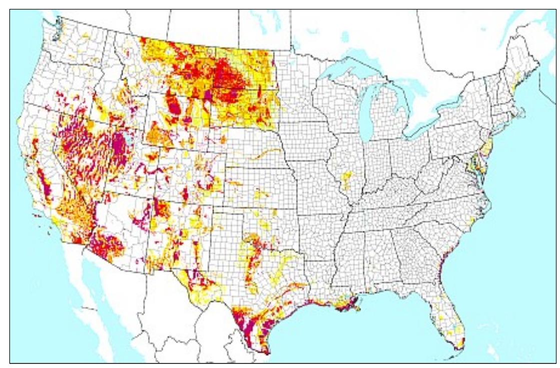
ABSTRACT

Developing alfalfa varieties with tolerance to salinity requires a multi-prong research approach. Because of the complexity of the plant by environment interaction a combination of laboratory, field and greenhouse selection techniques is most effective in addressing this complicated trait. At Forage Genetics our breeding strategy for salinity tolerance includes high throughput laboratory and greenhouse screens along with nursery and forage yield trials at multiple field sites with saline soils in CA, TX, WA, CO, SD and ID. Ion analysis of plant samples taken throughout the 2013 growing season from salt tolerant and salt susceptible plants in a salt nursery in SE WA, found differences in ion contents between salt tolerant and sensitive varieties. The correlation of K/Na ratio in alfalfa leaves and shoots may be an effective screening tool for salt tolerance.

Key Words: alfalfa, salinity, breeding.

INTRODUCTION

Soil salinity currently limits crop production potential in many parts of the western United States. The adjacent map by Biota of North America (2011) offers a high level regional view of saline soils in the U.S. Electrical conductivity (EC) is a commonly used measurement of salt content in soils. An EC>4.0 dSm-1 is usually used to describe a saline soil.



---

[1]Peter Reisen, preisen@foragegenetics.com, Forage Genetics International, 8770 Hwy 20/26, Nampa ID, 83687
[2]John Dodd, jdodd@foragegenetics.com, Forage Genetics International, 231 Second St., Touchet, WA, 99360
[3]Yun Kang, ykang@noble.org , Michael Udvardi, mudvardi@noble.org The Samuel Roberts Noble Foundation, 2510 Sam Noble Pkwy, Ardmore, OK, 73401
[4]Paul Tracy, pwtracy@landolakes.com 1080 Cty Rd. F, Shoreview, MN, 55156
[5]Amaia Seminario Huárriz, amaia.seminario@unavarra.esNatural Science Department, Public University of Navarra Campus de Arrosadía. 31006 Pamplona Spain
[6]Julie Ho, jho@foragegenetics.com, Forage Genetics International, 7661 Becker Rd., Davis, CA 95618
[7]Mark McCaslin, mmcaslin@foragegentics.com, Forage Genetics International , 1080 Cty Road F, Shoreview, MN, 55156

Soil salinization is often associated with application of irrigation water that is moderately high in salt content. Evaporation of water from the upper soil profile further concentrates salts near the soil surface. Another cause of saline soils is seen in the northern plains where salts are often introduced from a fluctuating water table and saline seeps bringing salts to the surface, which again concentrate via evaporation. Saline soils are most often found in arid regions where there is a lack of adequate rainfall to leach the accumulated salts down through the soil profile.

Saline soil conditions are often accompanied by two other soil problems: high sodium content (sodic soils) and high soil pH (alkaline soils). Sodic conditions negatively impact soil structure and water penetration, alkaline soils are suboptimal for plant growth per se, and limit availability of key nutrients. Soil type and clay mineralogy also play an important role in how a saline soil influences crop growth. The interaction of these conditions can cause a compounding stress on plants limiting crop establishment and crop production.

<u>Breeding alfalfa for salt tolerance</u>

The alfalfa research community has developed standard greenhouse tests designed to measure specific components of salt tolerance in alfalfa. A one week salt germination test (photo below) measures alfalfa seed germination under moderately high salt conditions and allows comparison of a new variety to established tolerant and non-tolerant checks.

Tolerant/Non-tolerant - salt germination test

A second greenhouse test measures plant growth under a defined high salt irrigation regime of 60mM NaCl. The six month test is designed to measure forage production potential under saline conditions (http://www.naaic.org/resource/stdtests.php).

Although these tests are designed to cover two independent and important components of salt tolerance, they are short in duration, lack the interaction with other associated stresses typical of sodic/alkalis soils, and are conducted in a greenhouse rather than field conditions. In an alfalfa study by Al-Niemi et al, 1992, the ability to germinate in high salt concentrations was found not to be related to post-germination growth and development under salt stress. Similarly a variety with high forage yield under saline vs. non-saline conditions can still be outperformed by a high-yielding variety that loses half of its yield when grown under saline conditions (Dewey, 1962). As best put by Flowers (2004): “Tolerance, judged in relative terms (i.e. yield in the saline conditions expressed as a proportion of yield in non-saline conditions), although an important indicator, is unlikely to impress a farmer unless absolute yield is adequate.”

In contrast Mott and Peel reported in 2010 that recurrent selection for two cycles using a greenhouse screen of a saline irrigation up to EC of 18.0 dS m-1 showed the top lines from one of the selected populations out yielding the check cultivars by thirty six percent.

To better understand the complexity of alfalfa growth and development in saline/sodic/alkali soils and to validate the efficacy of the greenhouse salt tests, we have identified field testing locations with saline soils in California, Colorado, Idaho, South Dakota, Texas and Washington. In a salt nursery establish in SE Washington with EC=21 dS m-1, pH=8.7 and very high sodium content (photo below) after two years >90% of first cycle plants were dead or dying with little difference between breeding lines selected for salt tolerance in greenhouse tests (selection range LD_{50} to LD_{75}) and those with no prior selection.

2012 Salt nursery SE Washington

Another promising breeding tool is measurement of the net accumulation of sodium, chloride and the potassium to sodium ratio of plants in salt nurseries. To test the potential of selection based on ion accumulation the SE Washington salt nursery was divided into four quadrants and the upper 4 to 6 inches of growth from tolerant and susceptible plants were sampled on three dates during growing season for net ion accumulation (Na, Cl, K, etc.). Of the ions tested, the K/Na ratio was most predictive in separating tolerant and susceptible genotypes suggesting it might be a useful selection tool (Table 1).

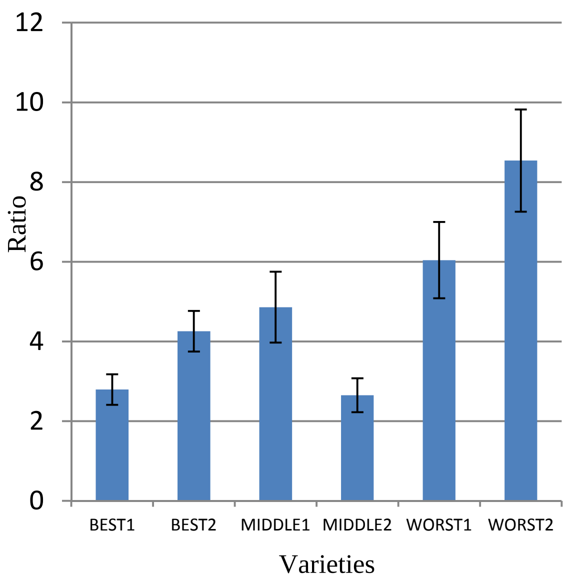
Table 1. Potassium to sodium ratio of salt tolerant and susceptible plants in the SE WA nursery.

K/Na index	04/10/13 mean	06/05/13 mean	10/02/13 mean
Tolerant	13.13	7.38	2.41
Susceptible	7.71	4.27	1.80
lsd (5%)	3.81	2.41	0.49
CV%	25.88%	25.37%	16.42%

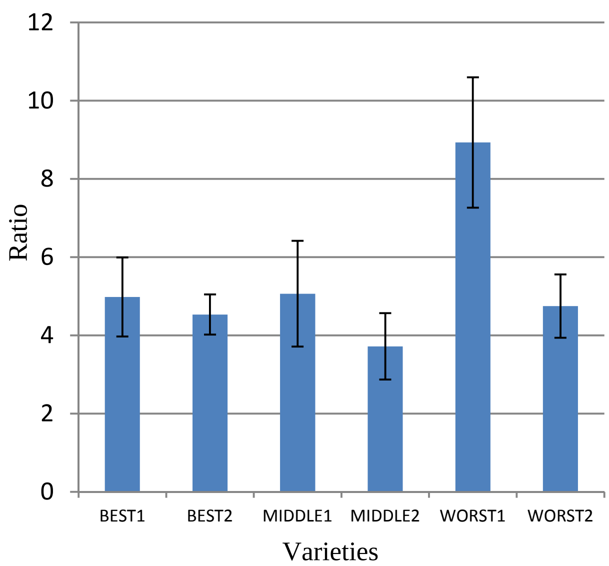
In another test to evaluate ion composition as a selection tool, tissue samples from plants of various salinity stress tolerance were collected at multiple times (May, July, and October) from our salt nursery in SE WA in 2013. The May and July samples have been analyzed for both cations and anions. Ion analysis in selected May and July samples indicated differences in ion contents between salt tolerant and sensitive varieties (Table 2). Repeatability of ion measurement of the same plant among different harvests was high. Future analysis will include the entire shoot samples in combination with growth chamber experiments for better identification of signature ions correlated with alfalfa salinity stress tolerance.

Table 2. Potassium/Sodium index in the SE WA nursery

May harvested alfalfa plants



July harvested alfalfa plants



Salinity improvement through transformation continues to show promise in multiple crops and numerous genes have been discovered that may play a role in salt tolerance (Flowers, 2012). One recent example is the AtNHX1 gene from *Arabidopsis thaliana* improving transgenic potatoes ability to tolerate NaCl at concentration levels 18 to 47 higher than the check varieties (Wang, 2013). Such progress is indeed encouraging. However it is important to keep in mind the extra cost in time and money incurred to a breeding program when using a transgenic approach. In a 2011 survey of six leading biotech companies, the estimated price and time for getting a transgenic trait to the market is $136M and 13.1 years (Phillip, 2011). For stacked traits, which are most common today, the cost and length of time to market increases further. For these reasons, a plant breeding company needs to look hard at balancing the time and costs of investments in developing a transgenic variety to the market demand before making a major research commitment.

Molecular markers associated with various components of salt tolerance are being developed that will increase our ability to select parents with favorable alleles and speed our breeding process. These high-precision and high-throughput assays will complement selection from our various salt nurseries and forage trials in developing new varieties with improved adaptation to problem soils.

Having compared various selection/plant breeding strategies for improving performance of alfalfa in typical saline/sodic/alkali challenged soils it appears the best strategy involves multiple cycles of field selection for vigor and yield combined with selection for ion exclusion and QTL/molecular markers selection.

Salt tolerant alfalfa varieties are but one tool in a grower's tool box to produce high quality hay in saline environments. Proper soil amendments, proper irrigation, etc. are also needed to maximize yield (see linked references below).

Useful Links

Managing Salt-affected Soils for Crop Production, D.A. Horneck, J.W. Ellsworth, B.G. Hopkins, D.M. Sullivan, and R.G. Stevens:
http://extension.oregonstate.edu/catalog/pdf/pnw/pnw601-e.pdf

Salinity Stress and its Mitigation, Rana Munns, Sham S. Goyal, and John Passioura:
http://www.plantstress.com/articles/salinity_m/salinity_m.htm

Managing Sodic Soils, J.G. Davis, R.M. Waskom, and T.A. Bauder* (5/12):
http://www.ext.colostate.edu/pubs/crops/00504.html

The use of saline waters for crop production - FAO irrigation and drainage paper 48:
http://www.fao.org/docrep/T0667E/T0667E00.htm

Irrigation Water Quality Standards and Salinity Management Strategies. Texas A&M Extension Service: http://soiltesting.tamu.edu/publications/B-1667.pdf

References

TS Al-Niemi, Campbell WF, and Rumbaugh MD. 1992. Response of alfalfa cultivars to salinity during germination and post-germination growth. Crop Sci. 32:976–980.

TJ Flowers. Improving crop salt tolerance. J. Exp. Bot. (2004) 55 (396): 307-319

P McDougall. 2011. Study: Cost of Bringing a Biotech Crop to Market http://www.croplife.org/PhillipsMcDougallStudy

IW Mott and Peel MD. NAAIC. 2010. Differential gene expression in salt-tolerant alfalfa (*Medicago sativa*). http://www.naaic.org/Meetings/National/2010meeting/Mott.pdf

Li Wang, Junlian Zhang, Di Wang, Jinwen Zhang, Yansen Cui, Yuhui Liu, Hongyu Yang, and Binyu. Crop Sci. 53:2643–2651 (2013). Assessment of Salt Tolerance in Transgenic Potato Carrying *AtNHX1* Gene.