What Have We Learned from 20 Years of Breeding for Alfalfa Root System Architecture?

--Zhanyou Xu,

Questions tested and to be tested

1. Can alfalfa root system architecture (RSA) be maintained across years and locations?
2. Which trait has the largest heritability to select for RSA breeding?
3. What has been the genetic gain of RSA breeding in the past 20 years?
4. Can machine learning replace the subjective selection with objective prediction?
5. Can RSA traits be commercialized? For example, $10 credit per acre by planting a specific RSA cultivar? CO_2 sequestration, soil erosion remediation, fertilizer runoff, drinking water contamination …

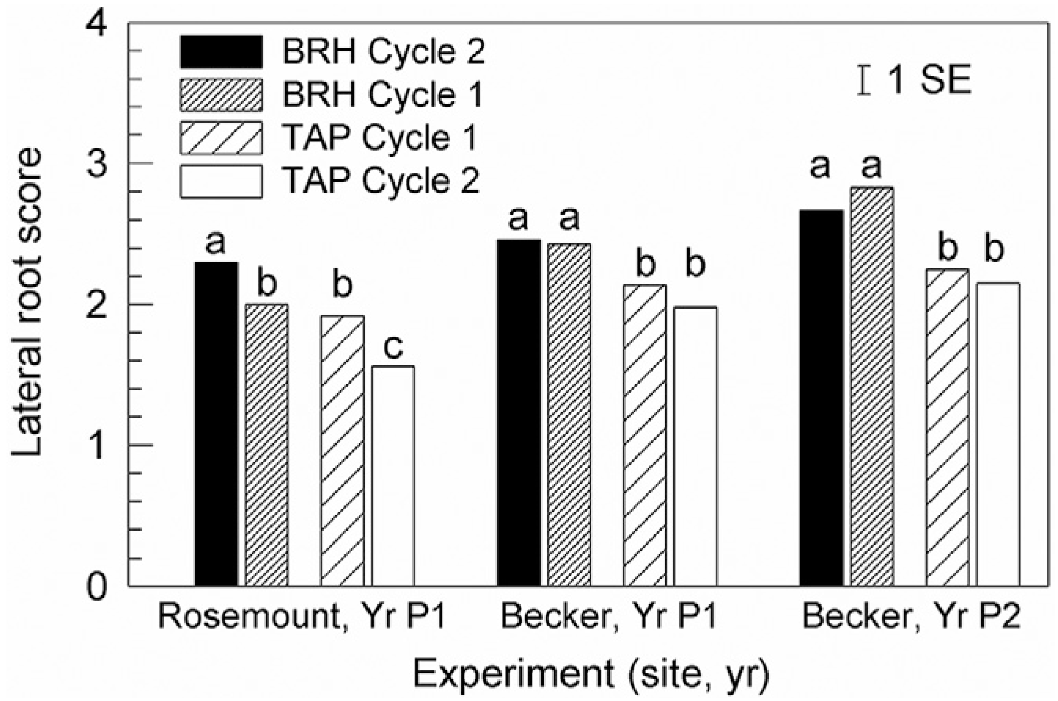
Agronomy Journal

Crop Ecology & Physiology | Full Access

Divergent Alfalfa Root System Architecture is Maintained across Environment and Nutrient Supply

Michael P. Russelle, JoAnn F. S. Lamb

First published: 01 July 2011 | https://doi.org/10.2134/agronj2011.0009 | Citations: 6

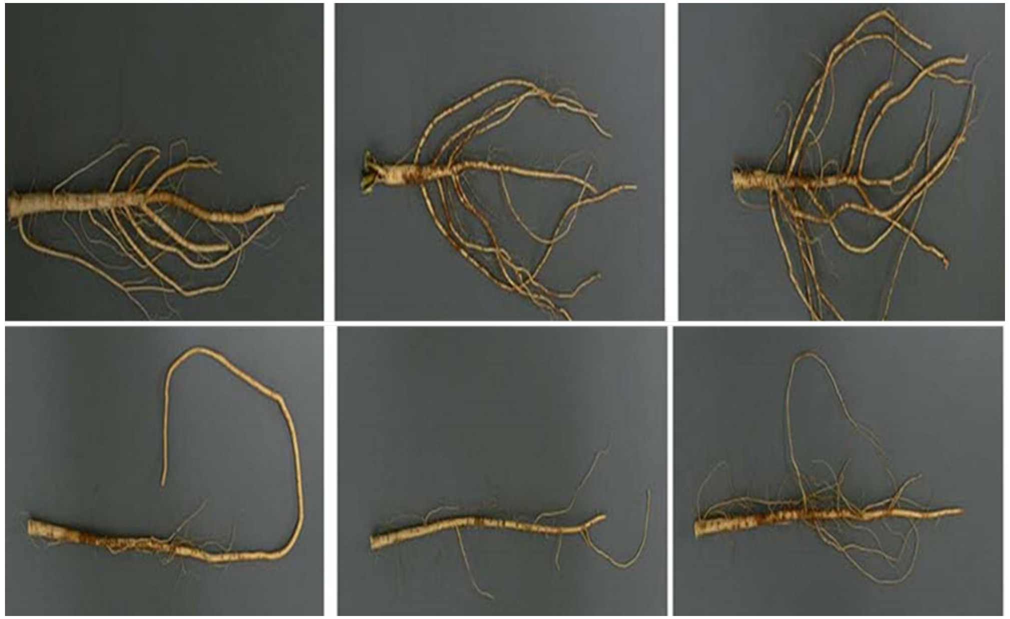


- **Confirmed**: The selected RSA traits were maintained across three years in two locations with different P supply and K placements.
- **Unsure**: Whether these differences in RSA confer improved agronomic or environmental benefits remains to be seen.
- **To test**: Future research should include more distinctly infertile soils, and differences in drought / wet soil tolerance should be evaluated.

Can alfalfa root system architecture (RSA) be maintained?

Yes

Which RSA trait has the largest heritability to select for root breeding?



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Plant Methods

Research | Open Access | Published: 07 December 2021

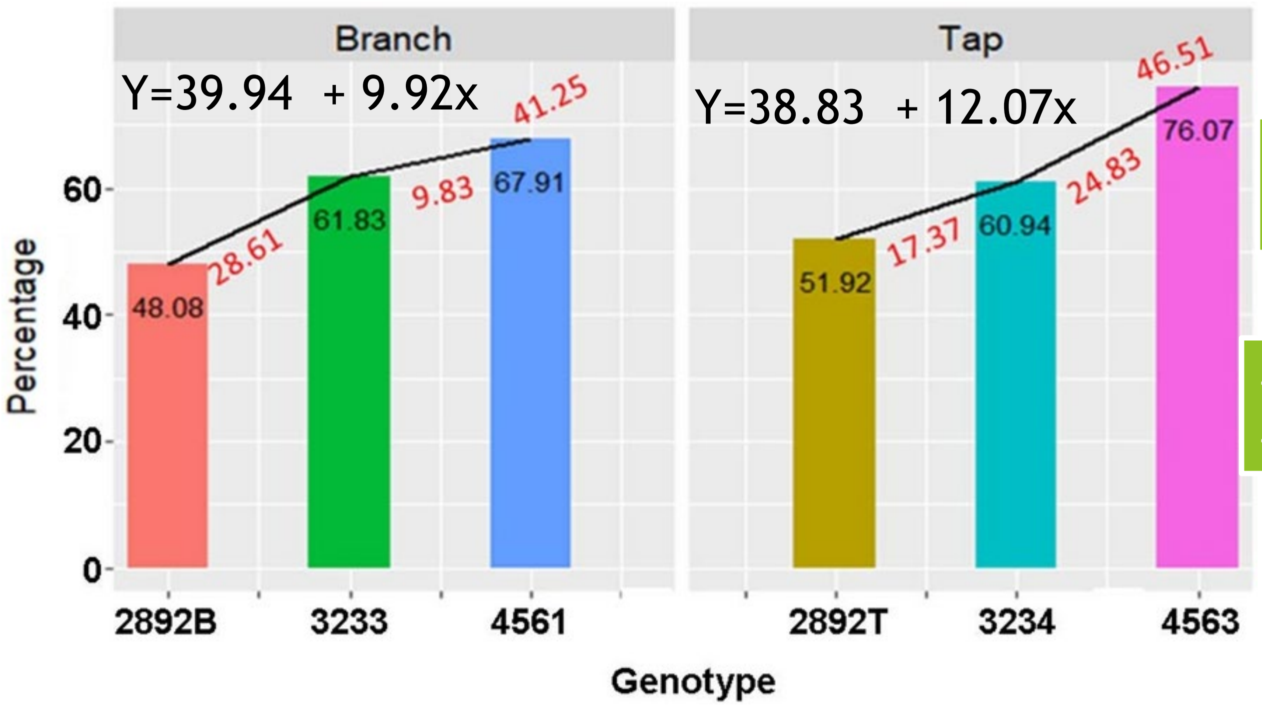
Phenotyping seedlings for selection of root system architecture in alfalfa (*Medicago sativa* L.)

Bruna Bucciarelli, Zhanyou Xu, Samadangla Ao, Yuanyuan Cao, Maria J. Monteros, Christopher N. Topp & Deborah A. Samac

Root parameter	Heritability
Total root length	0.59
Tap root length	0.00
Total lateral root length	0.60
Secondary root length	0.33
Tertiary root length	0.74
Secondary root number	0.00
Tertiary root number	0.79

- **Confirmed**: Tertiary root number is high and can be used for breeding.
- **Unsure**: Correlation of the root traits between early-stage 14-day-old seedlings and or matured (154 days) roots.
- **To test**: RSA performance at a commercial field planting density

RSA Genetic gain is higher than biomass



0.001/5.0= 0.02%

46.51%/4= 11.6%

0.08/4.5 = 1.78 %

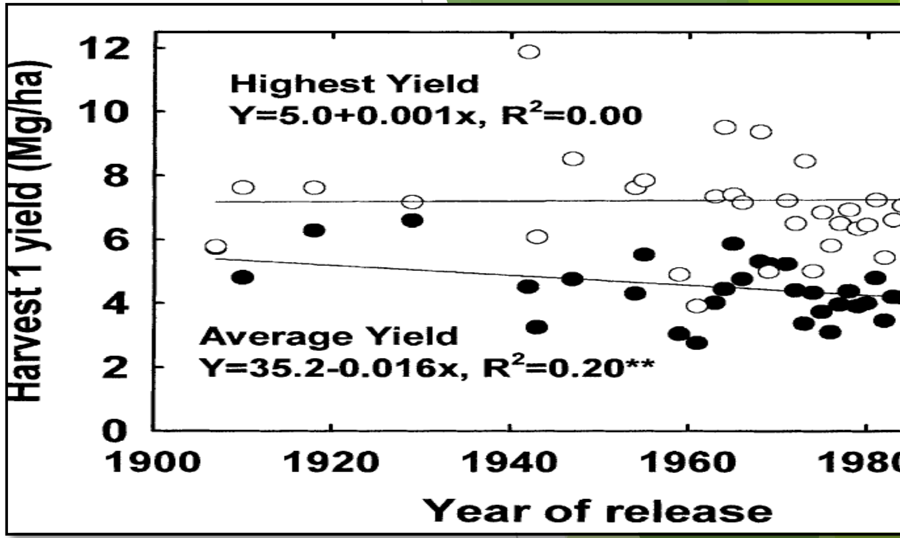


Figure from **Volenec**

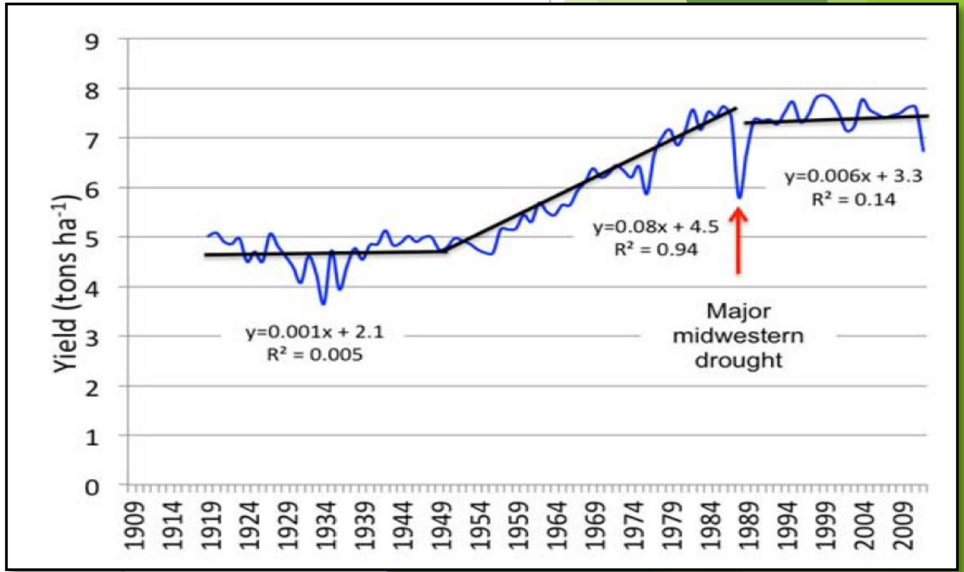


Figure from Brummer

What has been the genetic gain of RSA breeding in the past 20 years?

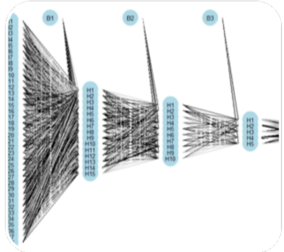
- The genetic gain from the breeding cycle is not 0

Phenotyping seedlings for selection of root system architecture in alfalfa (*Medicago sativa* L.) [Plant Methods]() **volume 17**, Article number: 125 (2021)

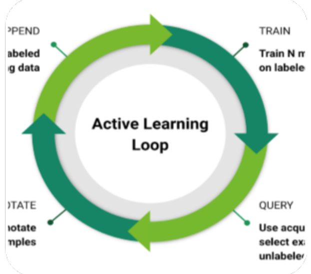
Can machine learning replace the subjective selection with the objective prediction?

Excavate the roots & take images

RhizoVision extract 38 traits

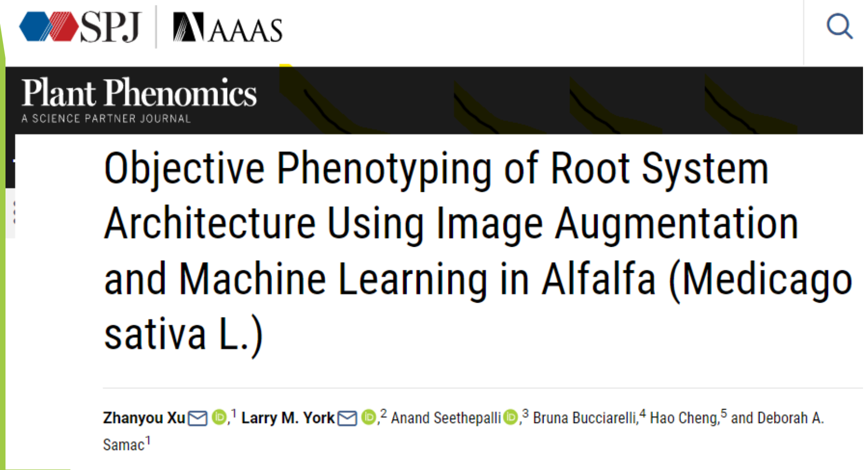


Unsupervised ML
Supervised ML
Deep Learning



Active ML
Probability

Neural network for objective phenotyping


SPJ | AAAS

Plant Phenomics
A SCIENCE PARTNER JOURNAL

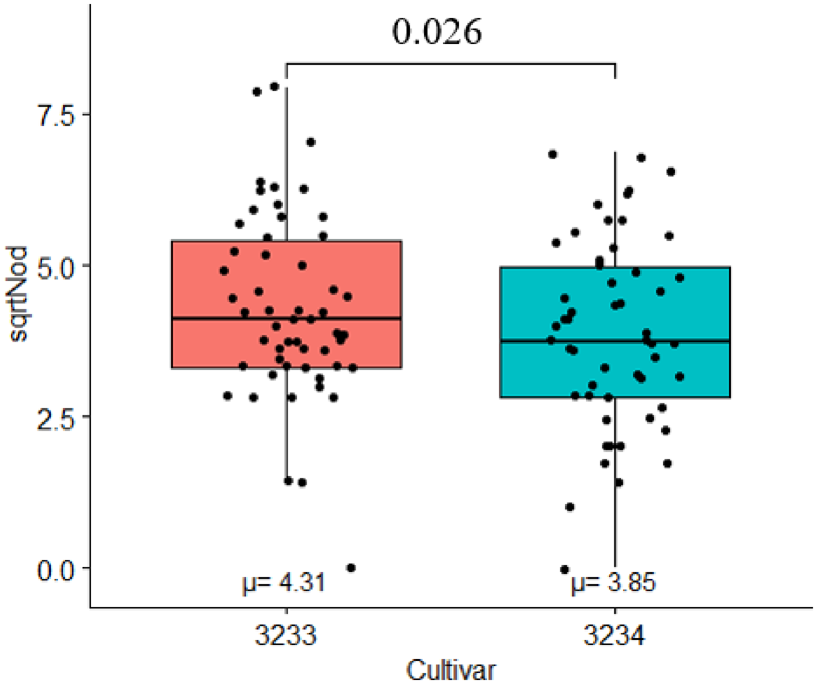
Objective Phenotyping of Root System Architecture Using Image Augmentation and Machine Learning in Alfalfa (Medicago sativa L.)

Zhanyou Xu[1], Larry M. York[2], Anand Seethepalli[3], Bruna Bucciarelli[4], Hao Cheng[5], and Deborah A. Samac[1]

	neural network (NN)			
Metrics	Branch	Taproot	TB	Mean
Prediction Accuracy	0.960	0.950	0.905	0.938

UMN3233, UMN4561

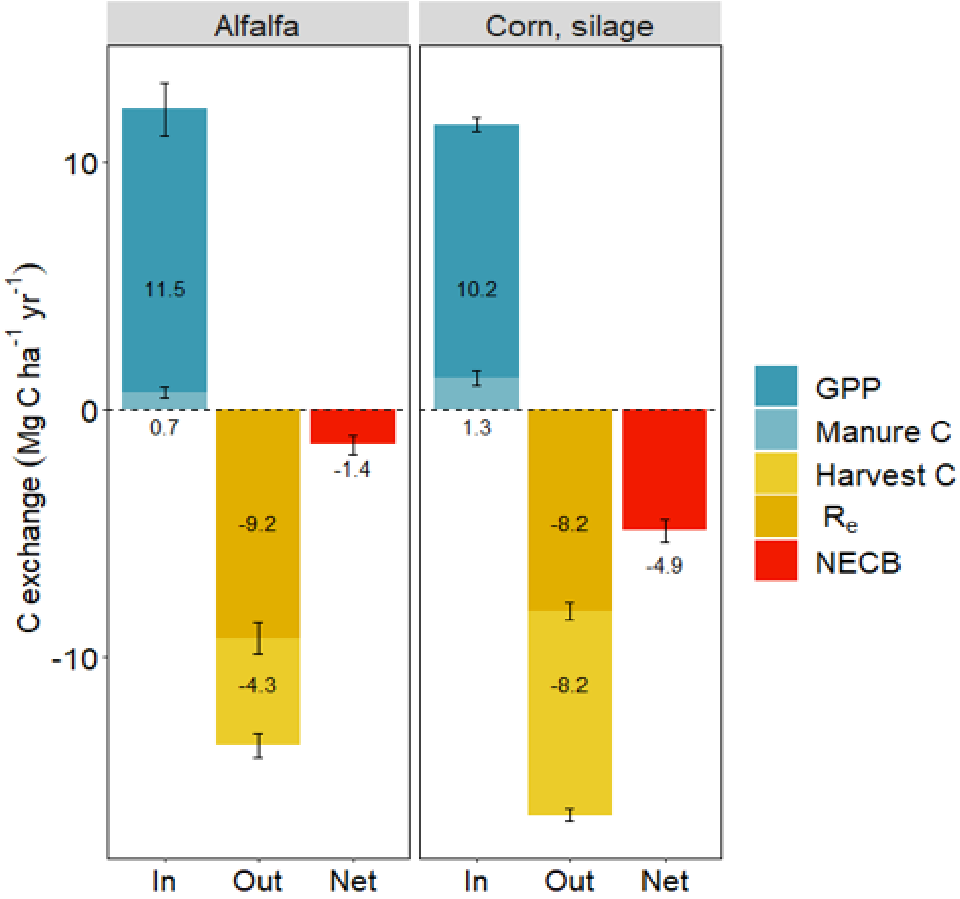
Square root of No of nodules



UMN3234, UMN4563

Can RSA be commercialized as a trait for the market? Nodulation differences

- **Confirmed:** Branch type has a significantly greater number of nodules
- **Unsure:** Will the branch type fix significantly more nitrogen?
- **To test:** Nitrogen fixation with ^{15}N



Can RSA traits be commercialized for the market? CO_2 Sequestration differences?

UMN4561

UMN4563

Agricultural and Forest Meteorology
Volume 306, 15 August 2021, 108438

Long-term ecosystem carbon losses from silage maize-based forage cropping systems

Joshua D. Gamble [a], Gary W. Feyereisen [b], Timothy J. Griffis [c], Chris D. Wente [d], John M. Baker [b]

- **Confirmed**: Alfalfa reduced C emission by 23% relative to continuous silage maize.
- **Unsure**: RSA difference among cultivars is significant enough for CO_2 sequestration.
- **To test**: C storage by branch vs. tap root populations.

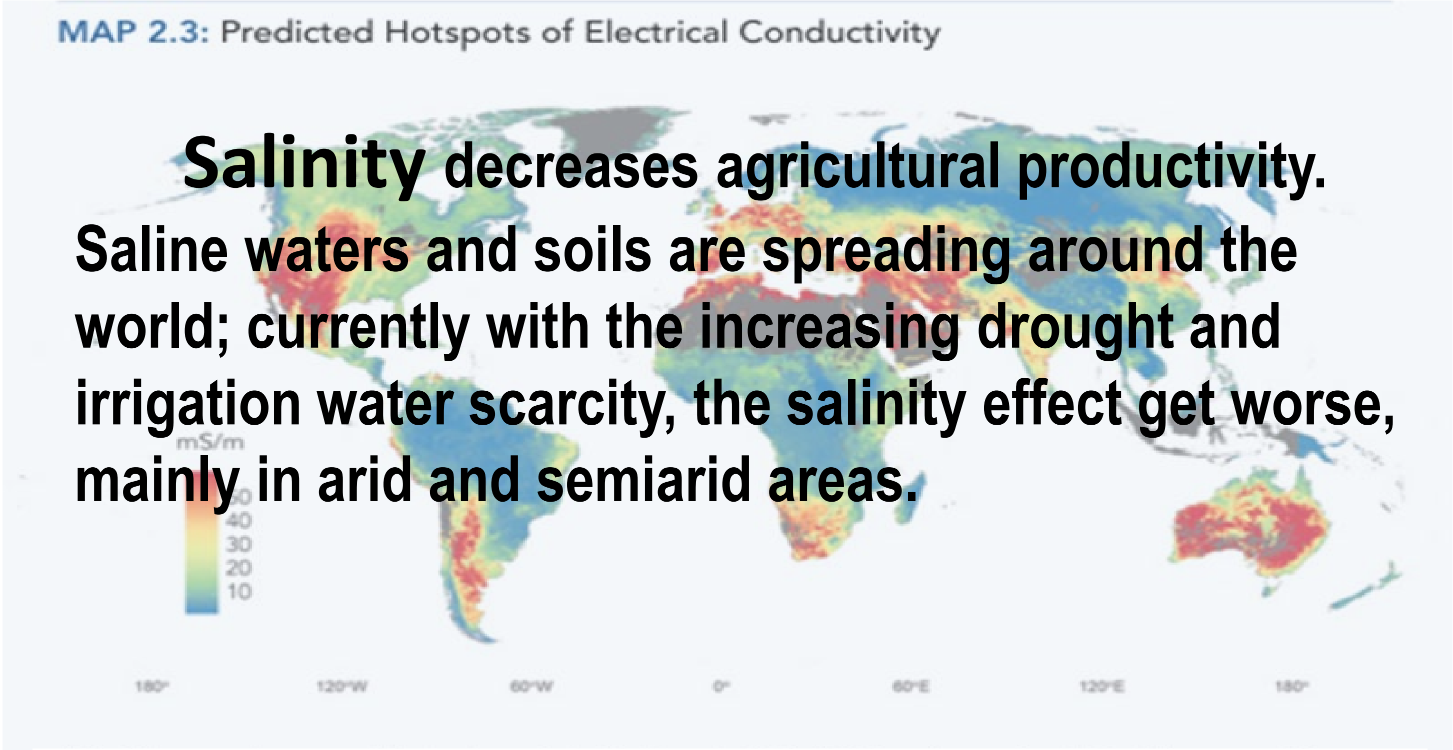
Acknowledge

- Zhanyou Xu, Deborah A. Samac, and Bruna Bucciarelli, Josh Gamble, Samadangla Ao from (USDA)
- Larry M. York (ORNL) and Anand Seethepalli (NRI)
- Maria J. Monteros from Noble Research Foundation, now Bayer
- Christopher N. Topp from Donald Danforth Plant Science Center,
- Hao Cheng (US Davis)

Salinity effects on the performance of alfalfa populations in a semiarid enviroment of Argentina

2022 WORLD ALFALFA CONGRESS SAN DIEGO CA | USA
November 14-17, 2022

Mónica Cornacchione and Salvador Prieto Angueira
National Institute of Agricultural Technology, Santiago del Estero, Argentina.



Salinity decreases agricultural productivity.

Saline waters and soils are spreading around the world; currently with the increasing drought and irrigation water scarcity, the salinity effect get worse, mainly in arid and semiarid areas.

"Damania, et al. 2019. Quality Unknown: The Invisible Water Crisis.
https://openknowledge.worldbank.org/handle/10986/32245

Alfalfa

Importance globally

- ✓ Recent research has shown that alfalfa is more tolerant to salinity (*Cornacchione and Suarez, 2015, 2017; Putnam et al, 2017; Benes et al, 2018, etc*) than previous studies with oldest varieties.

potential to contribute to the sustainability of semiarid regions

Evaluate the alfalfa populations under natural saline conditions

Santiago del Estero, NW **Argentina**
high TºC and long warm season
pp (2019-2021): ~500 mm/year

Salinity

pre sowing /before raining season/ final

EM38 to assess the spatial and temporal variability in soil salinity

EM-38 instrument

① **Apparent -ECa**

② **Extracted soil -ECex**

③ **Estimated -ECes**
for each plot

Populations

Ameristand801 **(AME)**
Salado **(SDO)**

MSI0036 **(M36)**
MSI0037 **(M37)**
MSI0038 **(M38)**

Chenini **(CHE)**

Salina PV **(SNA)**
Kumen PV INTA **(KUM)**
Salinera INTA **(SRA)**
Monarca **(MON)***
PISuperMonarca **(SMO)***

Sardi **(SAR)***

Sowing (fall 2019)
Thinning (spring 2019): 55pl/m^2

water: establishment and winter and early spring 2020.

Experimental design:

Latinized row-column (4x3), three rep.

Biomass production (16 cuts), survival

Statistical analysis

ANOVA using GLM model and **ECes as a covariable.** AP means were compared using the LSD Fisher test (P<0.05).

Salinity TEMPORAL variability

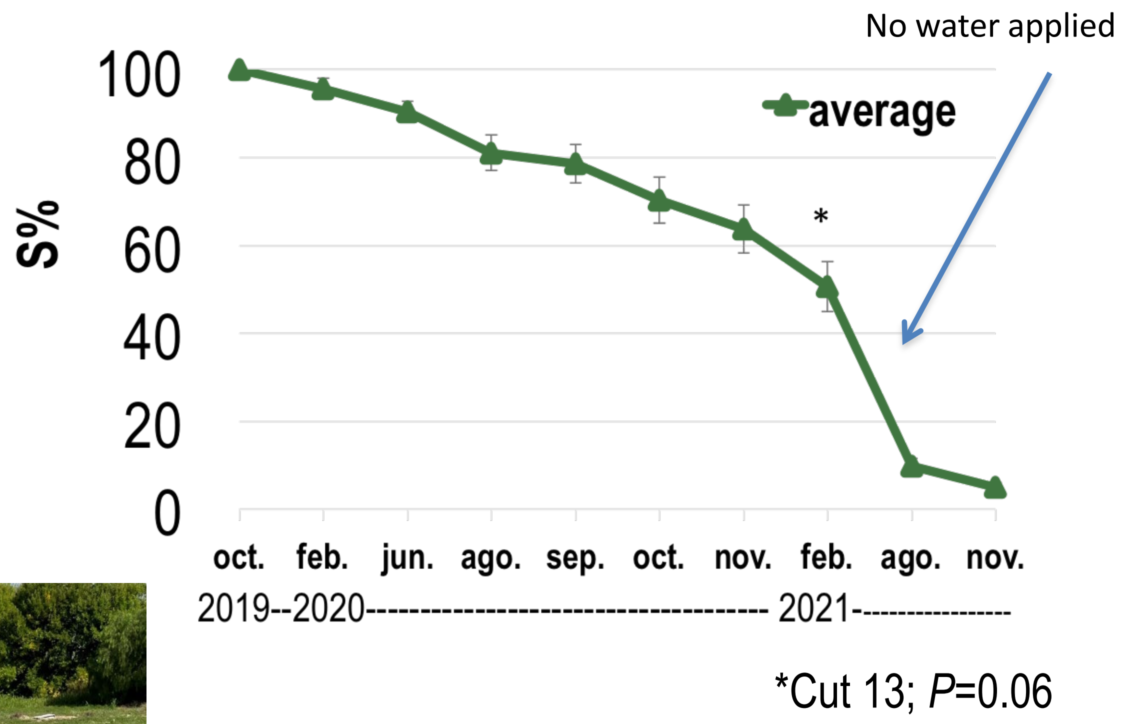
Average ECes dS/m

Depth (cm)	Pre-sowing March 2019	Dec. 2020	Oct. 2021
0-90	9.5	27.4	25.8
range	6.0 - 13.5	22.0 - 33.1	23.4 - 29.2

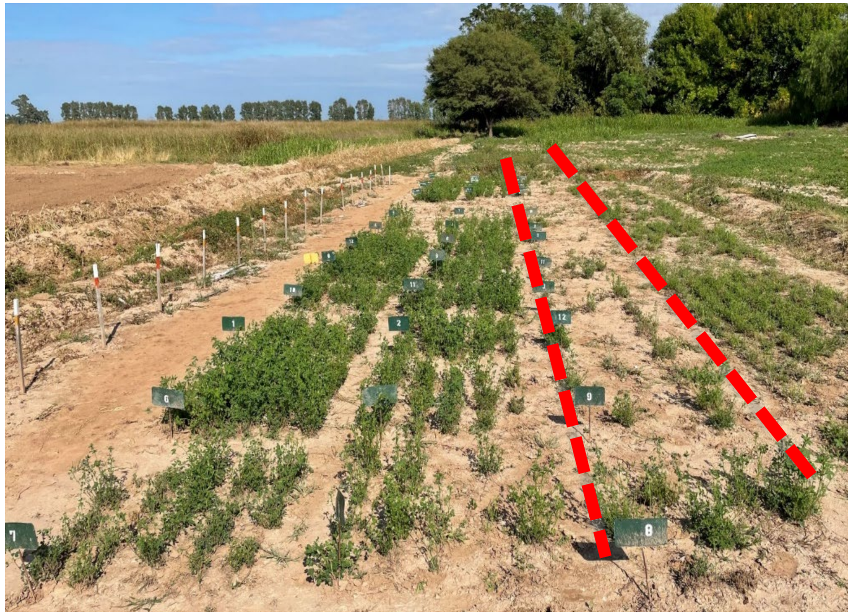
pH 6.7 to 7.4

COVARIABLE

Relative survival of plants

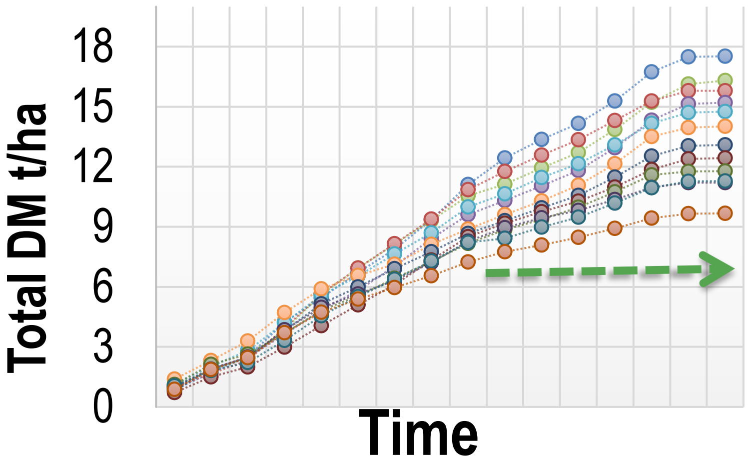


SPATIAL variability

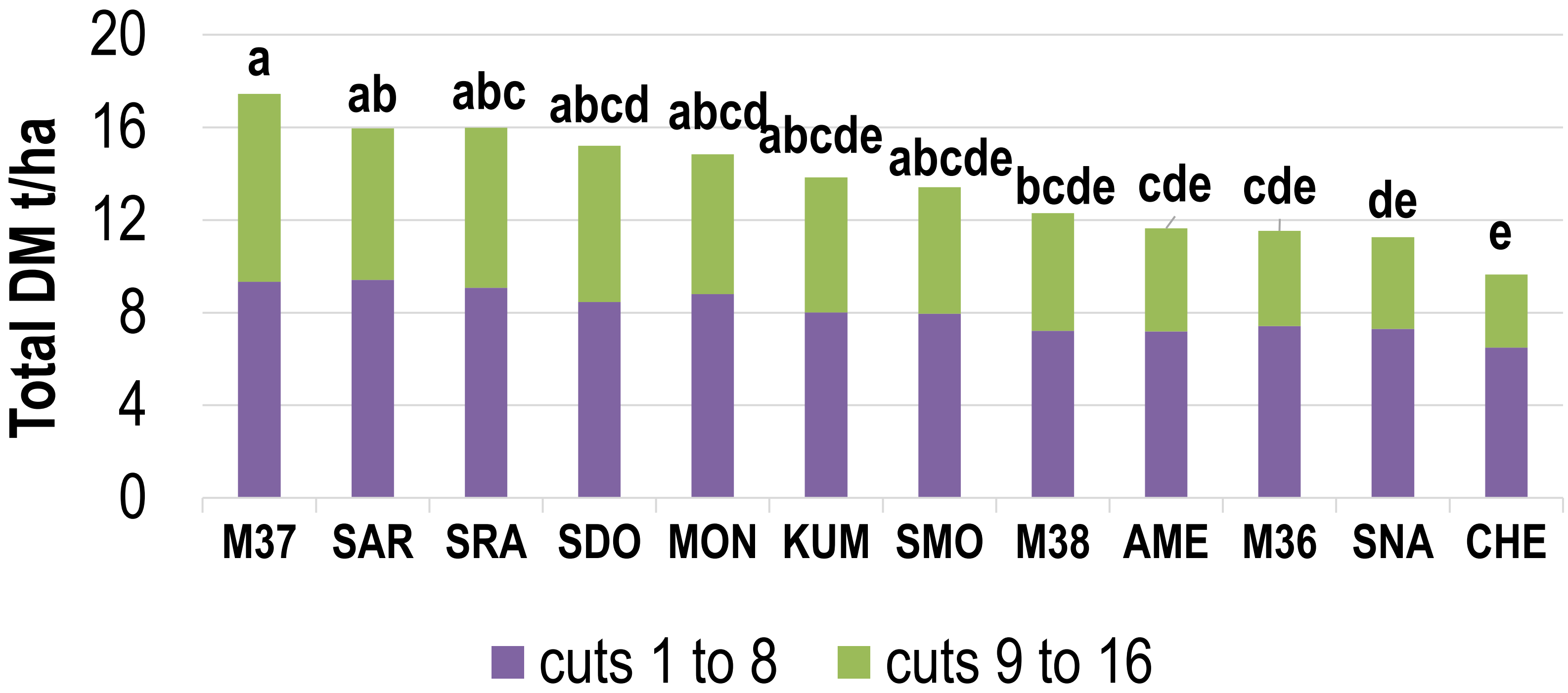


Noticeable loss of plants on the right side of the trial -column 3- (date: april 2021)

Biomass production



first 8 cuts (*P*>0.05): ~9.5 to 6.5 t/ha (pp + irrigation)
second 8 cuts (*P*<0.05) : ~8 to 3 t/ha (pp)



Total production (*P*<0.05) 2019/2021, 16 cuts: ~17.5 to 9.5 t/ha

Our results suggest that when **soil salinity increased to values around 20 dS/m,** the AP displayed a different aptitude to cope with this stress, which also was more stressful without irrigation.

the emergence and establishment took place with lower EC

Even though screening populations in the field is difficult due to the high heterogeneity, this study increases the knowledge about both the decrease and variability of alfalfa production under saline conditions.

Many thanks

Salinity Tolerance and Forage Quality Comparison of Four Varieties Of Alfalfa (*Medicago* sativa L.)

Victoria De Leon, Ranjit Riar, Sharon Benes and Anil Shrestha,
California State University, Fresno

Study Objectives

Irrigation water is becoming scarce due to increasing drought. Many of California’s Central Valley specialty crops (almonds, citrus, grapes and other vegetables) are salt-sensitive.

Forages such as alfalfa will increasingly be irrigated with saline waters such as agricultural drainage, wastewaters from animal production and food processors, or reclaimed municipal waters

This study’s objective is to evaluate the salt tolerance of 2 new commercial varieties of alfalfa in comparison to a salt-tolerant (AZ90NDCST) and a public (CUF101) control under outdoor conditions (large pots, 60:40 clay loam soil and sand).

Trial conducted at request of seed company

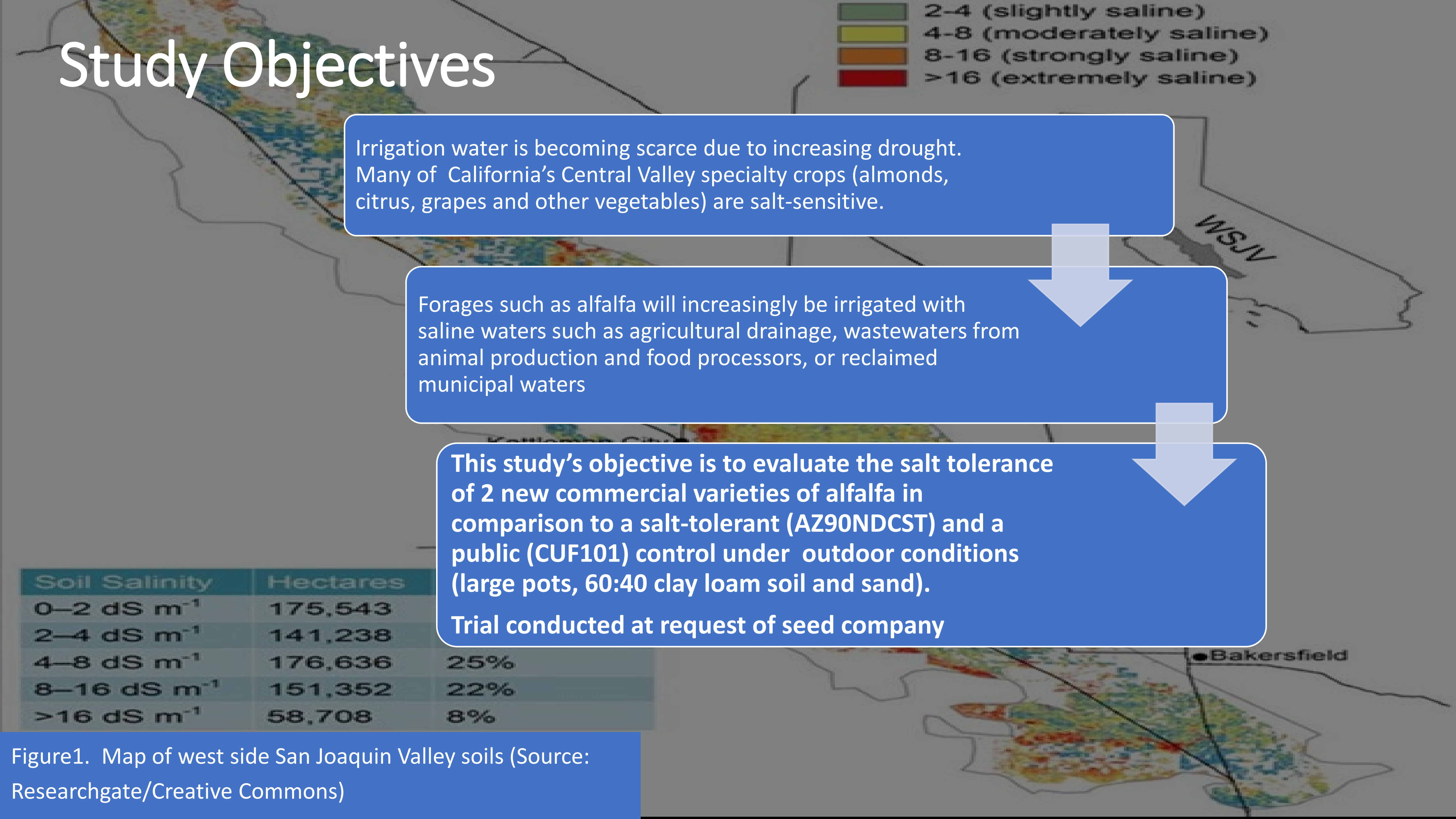
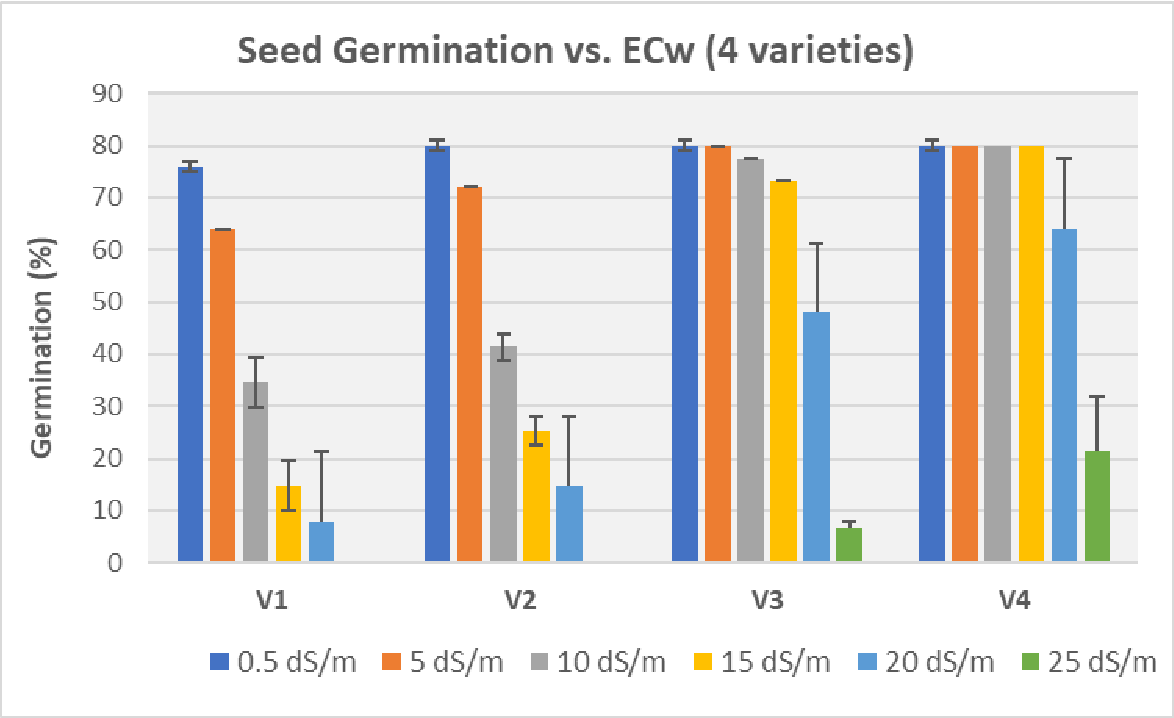


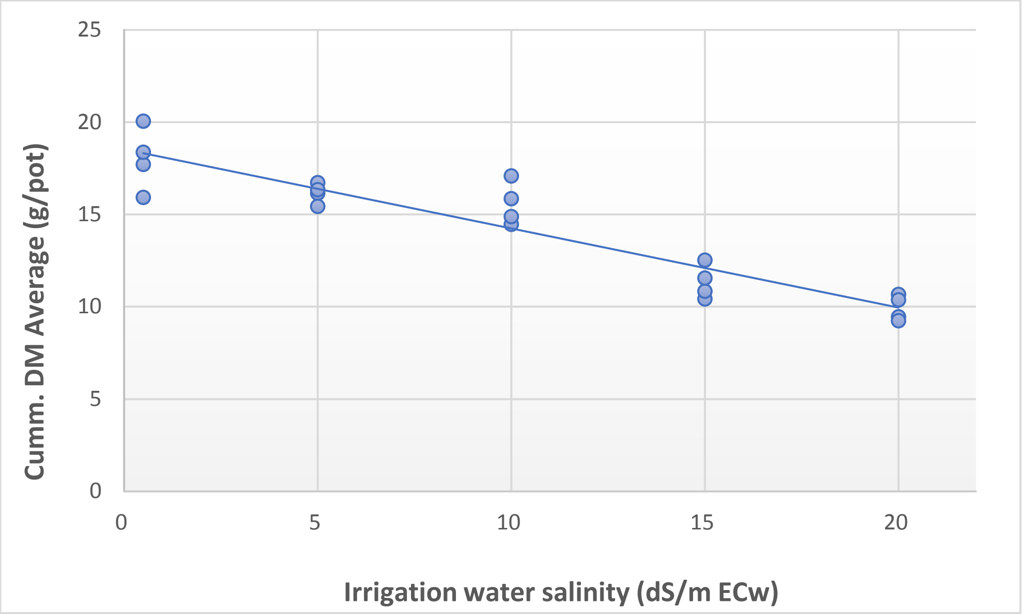
Figure1. Map of west side San Joaquin Valley soils (Source: Researchgate/Creative Commons)

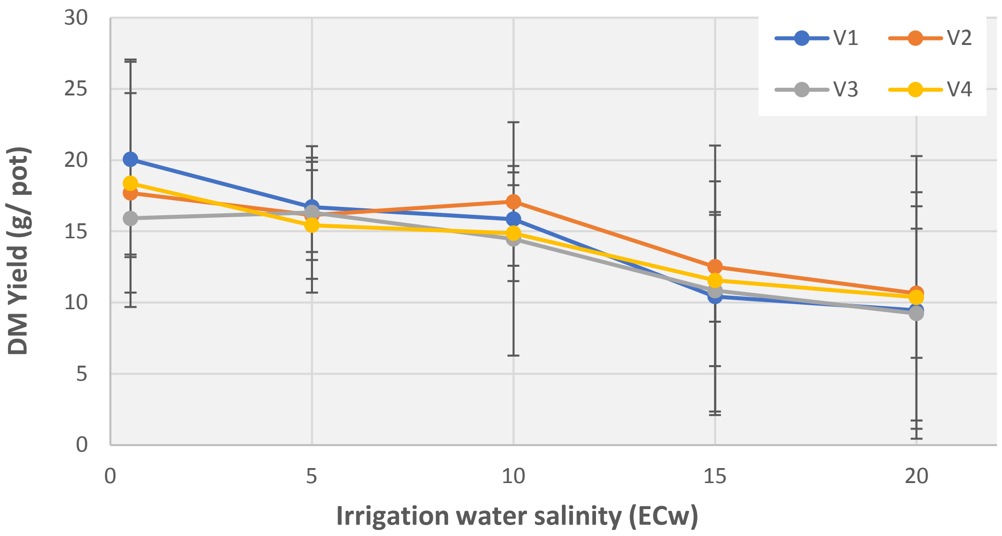
Seed Germination (%- average of 3 trials)



Salinity	V1	V2	V3	V4
0.5	76.0	80.0	80.0	80.0
5	64.0	72.0	80.0	80.0
10	34.7	41.3	77.3	80.0
15	14.7	25.3	73.3	80.0
20	8.0	14.7	48.0	64.0
25	0.0	0.0	6.7	21.3

- Varieties 1 and 2 showed a steady decrease in germination percentage as salinity of the irrigation water (ECw) increased
- **Variety 4 was the most salt tolerant at germination** maintaining maximum germination (80%) even at 15 dS/m ECw
- Varieties 3 and 4 were more salt tolerant at germination, maintaining 73 to 80% germination at 15 dS/m, as compared to 15 to 25% germination for varieties 1 and 2.





Cumulative Dry Matter Yield (7 cuts)

Effect of irrigation water salinity was highly significant (P = 0.0002)

Variety was not significant (P = 0.1879), nor was the Variety x Salinity interaction (P = 0.6383)

- Shoot DM yield decreased quite uniformly as salinity of the irrigation water increased from 0.5 to 20 dS/m ECw
- Varieties did not show large differences in absolute dry matter yield even at the higher salinities
- However, based on relative yield (RY) = yield in comparison to nonsaline control (0.5 dS/m),
  - **10 dS/m ECw: 3.5 to 20.9% yield loss**
  - **15 dS/m Ew: 29.3 to 48% yield loss**
  - **20 dS/m Ecw: 39.8 to 52.9% yield loss**

Absolute Yield

Salinity	V1	V2	V3	V4
0.5	20.1	17.7	15.9	18.4
5	16.7	16.1	16.3	15.4
10	15.9	17.1	14.5	14.9
15	10.4	12.5	10.8	11.6
20	9.4	10.7	9.2	10.4

Relative Yield (compared to 0.5 dS)

RY 5	83.3%	91.2%	102.6%	84.0%
RY 10	79.1%	96.5%	90.9%	80.9%
RY 15	52.0%	70.7%	68.1%	62.9%
RY 20	47.1%	60.2%	58.1%	56.5%

Conclusions

Based on data evaluated thus far…

- Variety 4 was the most tolerant at seed germination
- **Varieties 3 and 4 were more salt tolerant at germination than were 1 and 2**
- Varieties did not show much difference in cumulative dry matter yield DM yield even at the higher salinities and the effect of variety was not significant.
- **Based on relative yield** (comparison to non-saline treatment), **varieties 2 and 3 were the most salt tolerant.**
- **Variety 3 (one of two new commercial varieties) is numerically superior** to the public (CUF101) and salt tolerant (AZ90NDCST) control based on the combination of germination and relative dry matter yield under highly saline irrigation
- All varieties maintained nearly 80% of dry matter production at irrigation water salinities up to 10 dS/m ECw. However, the outcome of saline irrigation can differ considerably based on soil texture and irrigation volume and frequency

Overall Conclusions and future work

- As found in other studies (Cornacchione and Suarez 2015, 2017) and Benes et al. (2018), alfalfa is much more salt tolerant than established guidelines indicate
- Na and K levels and forage quality will be compared for the 4 varieties at each salinity level.
- Dry matter yield outcome will be evaluated based on soil salinity once data are available