Response of *Camelina V*arieties to NaCl Salinity

By Ms. Monica Effi

Mentor: Dr. Josekutty

Discussion Paper

Camelina Production in Montana

McVay, K. A. *Montana State University Extension - Bozeman Montana*. Mar. 2008

- A member of the mustard family, camelina is an oilseed crop that has demonstrated better drought tolerance and greater spring freezing tolerance than canola.
- It appears resistant to flea beetles, a major economic pest of canola in Montana environments.
- It has potential for successful dryland production across Montana (it should be a good choice for rotating with small grain crops).

Crop History and Adaptation

- Camelina [*Camelina sativa* (L.) Crantz., Brassicaceae] is native to Central Asia and the Mediterranean with both annual and winter annual biotypes.
- Camelina plants are heavily branched, growing to heights of one to three feet. They produce prolific small, pale yellow or greenish-yellow flowers consisting of four petals.
- It is a short-season crop, best adapted to cooler climates where excessive heat during flowering does not occur.
- Its drought and spring freezing tolerance may make it a good fit for areas in Montana where canola production is difficult.

Camelina oil has good potential for food and industrial use

- Unique properties of camelina oil could lead to development of a wide array of high value markets for the oil and its components in foods, feeds, cosmetics and industrial products.
- Nutritional: increased nutritional value of a range of baked food such as bread.
- Biodiesel: biodiesel performance appears to be equal in value and indistinguishable from biodiesel produced from other oilseed crops such as soybean.

Agronomic Research in Montana

- Camelina has just recently been introduced in Montana. Cultivar selection and agronomic trials continue to be conducted to determine proper planting rates, planting dates, fertility practices, and refinement of management techniques to improve overall growth of the crop.
  - Field selection and rotation: it is critical to select fields where prior management has limited weed pressure and weed seed production was kept to a minimum. Recommendation for seeding camelina is to follow plant-back restrictions specified for canola on herbicide labels.

Contd.

- Seeding method: current recommendations are to drill camelina seeds very shallow utilizing backer wheels to ensure good seed to soil contact and a firm seedbed.
- Harvest: Harvest dates vary from late June to late July depending on seeding date, precipitation, temperature, and harvest method.
- Seed storage: seeds are very susceptible to damage from high moisture conditions. They should be stored at 8 percent or lower moisture.

What's Next?

- Early experience in Montana has shown that with good management, and timely planting, good crop yields can be attained.
- Due to constraints of the oil seed crushing industry, camelina production in the near future will only be grown under private contract so that enough seed for a crushing run can be accumulated by the contracting company.
- Other high value uses of camelina and omega-3 oil is still being researched.

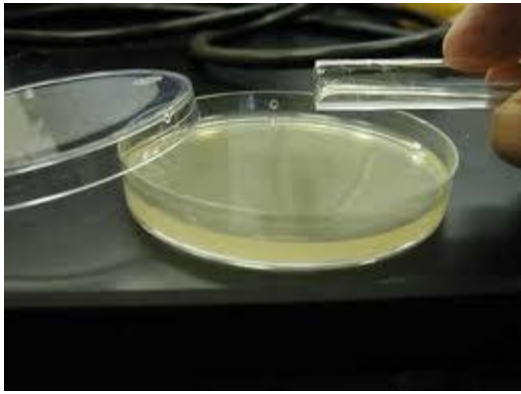
THE EFFECT OF SALINITY (NaCl) ON GROWTH AND ACCUMULATION OF PROLINE IN *Camelina sativa GROWN IN VITRO.*

Introduction

- Salinity is a major constrain for crop production
- Proline is an amino acid that helps plants to combat abiotic stress such as cold, drought and salinity. Therefore, genotypes that can accumulate greater proline under stress may have greater stress tolerance.
- We are comparing three genotypes for their growth and proline accumulation under salt stress

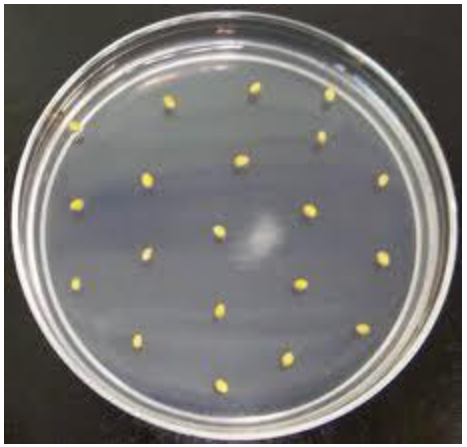
Materials and methods

- We used MS (Murashige and Skoog 1962) medium supplemented with 50-300 mM NaCl for growing seeds *in vitro.*Medium (different volumes as needed) was prepared as follows:
- Added 4.43 g/l of MS medium powder
- Added 20g/l sucrose
- Added necessary amounts of NaCl to make 50-300 mM conc. in the medium
- Adjusted the pH to 5.8
- Added 7g/l agar
- Medium was sterilized for 20 min. at 121°C
- Dispensed 25ml medium to petri dish
- Sealed, labeled, and stored the plates at 4°C until used



In vitro culture technique

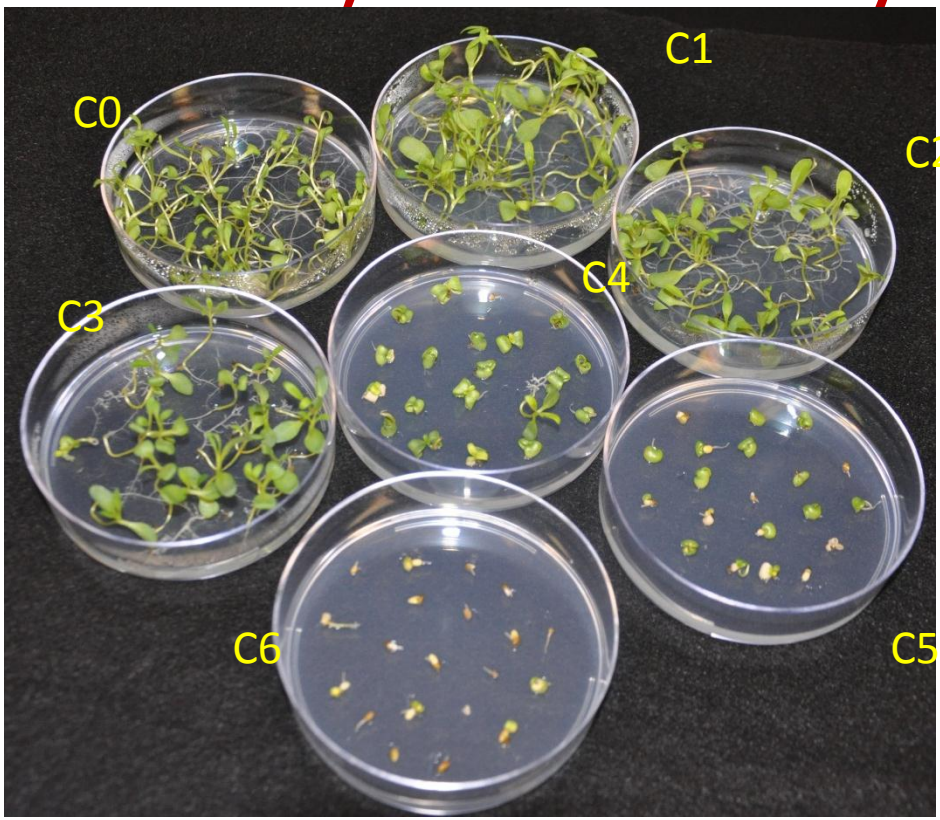
- We used *C. sativa* cv. Blain Creek, Suneson and Cheyenne in this experiment
- Decontaminated the seeds through following method and inoculated onto the medium
- Washed for 10 minutes with a drop of tween 20®
- Treated with 70% ethanol for 5’
- Rinsed once with sterile water
- Treated with 25% bleach for 10’
- Rinsed three times with sterile water
- Inoculated 20 seeds per plate
- Incubated in the dark for 2 days
- Incubated in the lighted racks till analyzed for proline content on day 21using the protocol (Bates et al, 1973)



Determination of rate of germination, growth and proline content

- *Germination data* was collected on day 2 and after a week from culture
- *Growth* was determined as increase in fresh weight (dry weight is being gathered)
- *Determination of proline content using Bates et al. 1973*
- Ground 0.5g tissue in 5 ml 3% sulfosalysilic acid, filter
- Mixed 2 ml filtrate with 2 ml nynhydrin reagent and 2 ml glacial acetic acid, heated in a boiling water bath for an hour
- Cooled in an ice bath, added 4 ml toluene, separated the chromosphere in the toluene fraction, read absorbance at 520 nm with a spectrophotometer
- Prepared standard graph using 10,20,40, 80 and 100 µg/ml of proline. Calculated proline content of the unknown samples with the help of the standard graph
- Calculated ug proline/ g fresh weight (Fr Wt.) using the formula (***µg/ml proline x vol. of toluene x volume of SS acid)/ (g Fr. Wt x 115.5***)

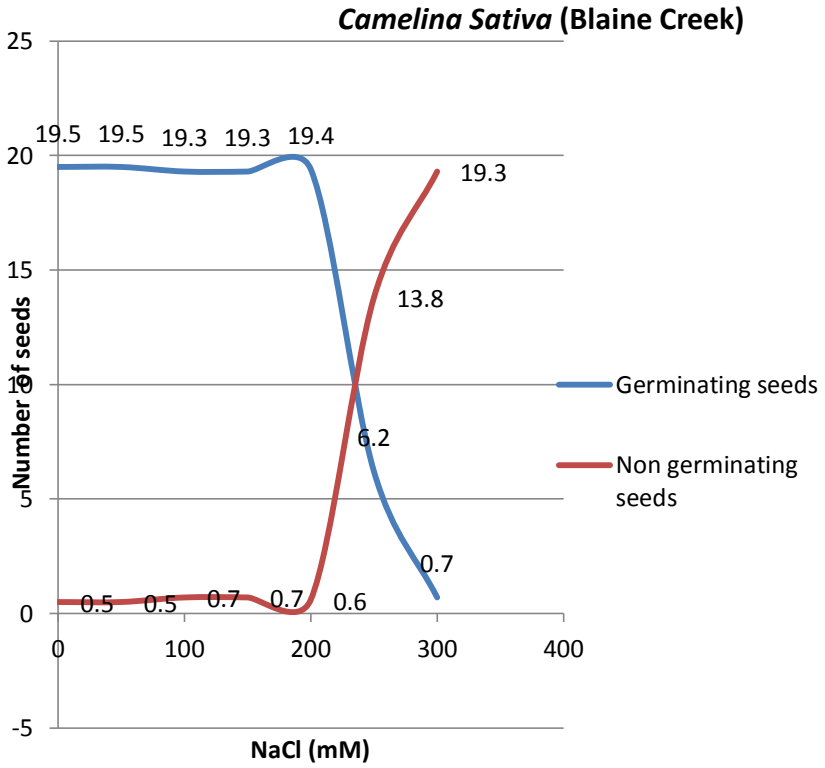
Seedlings of *Camelina sativa* (Blaine Creek) after 21 days



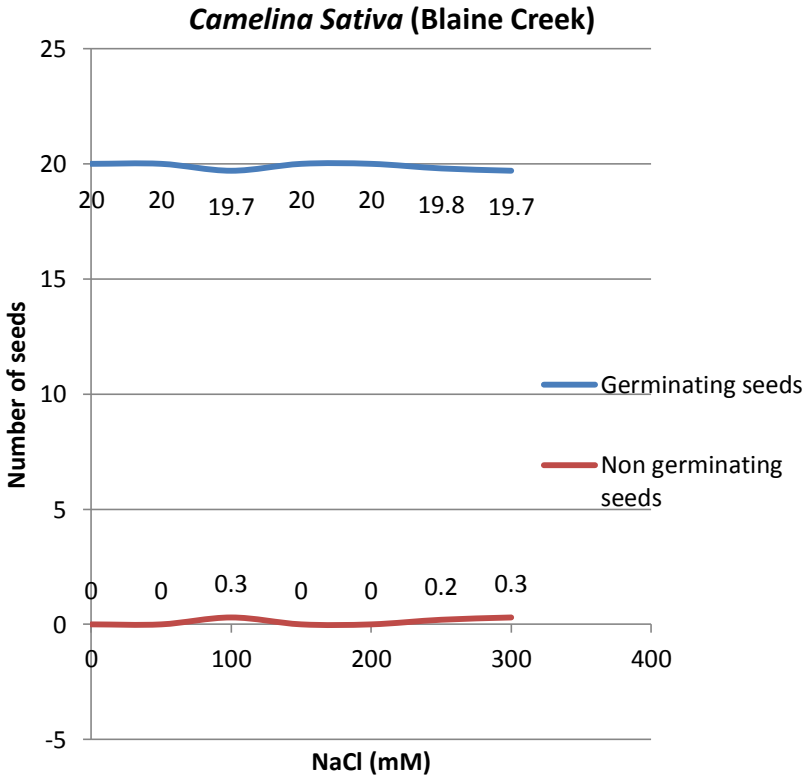
C0 (0 mM), C1 (50 mM), C2 (100 mM), C3 (150 mM), C4 (200 mM), C5 (250 mM), C6 (300 mm) NaCl

Germination of cv. Blaine Creek seeds

After 2 days

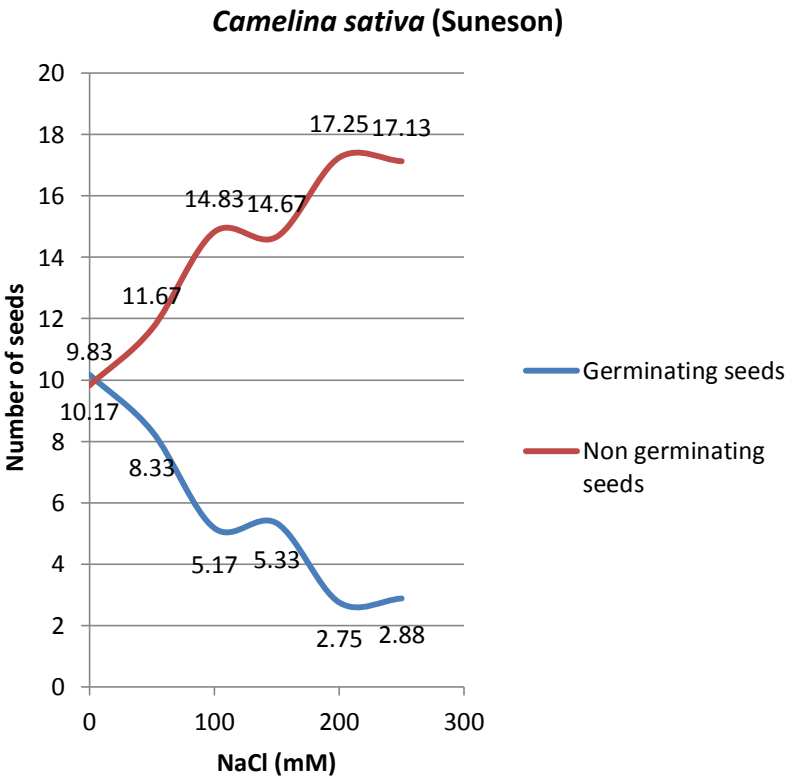


After 10 days

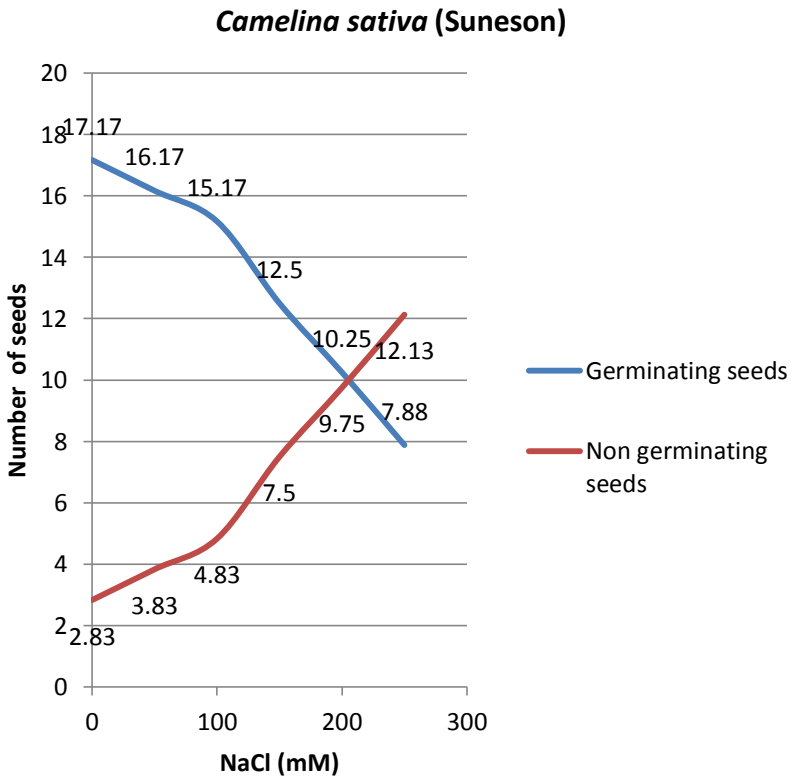


Germination of cv. Suneson seeds

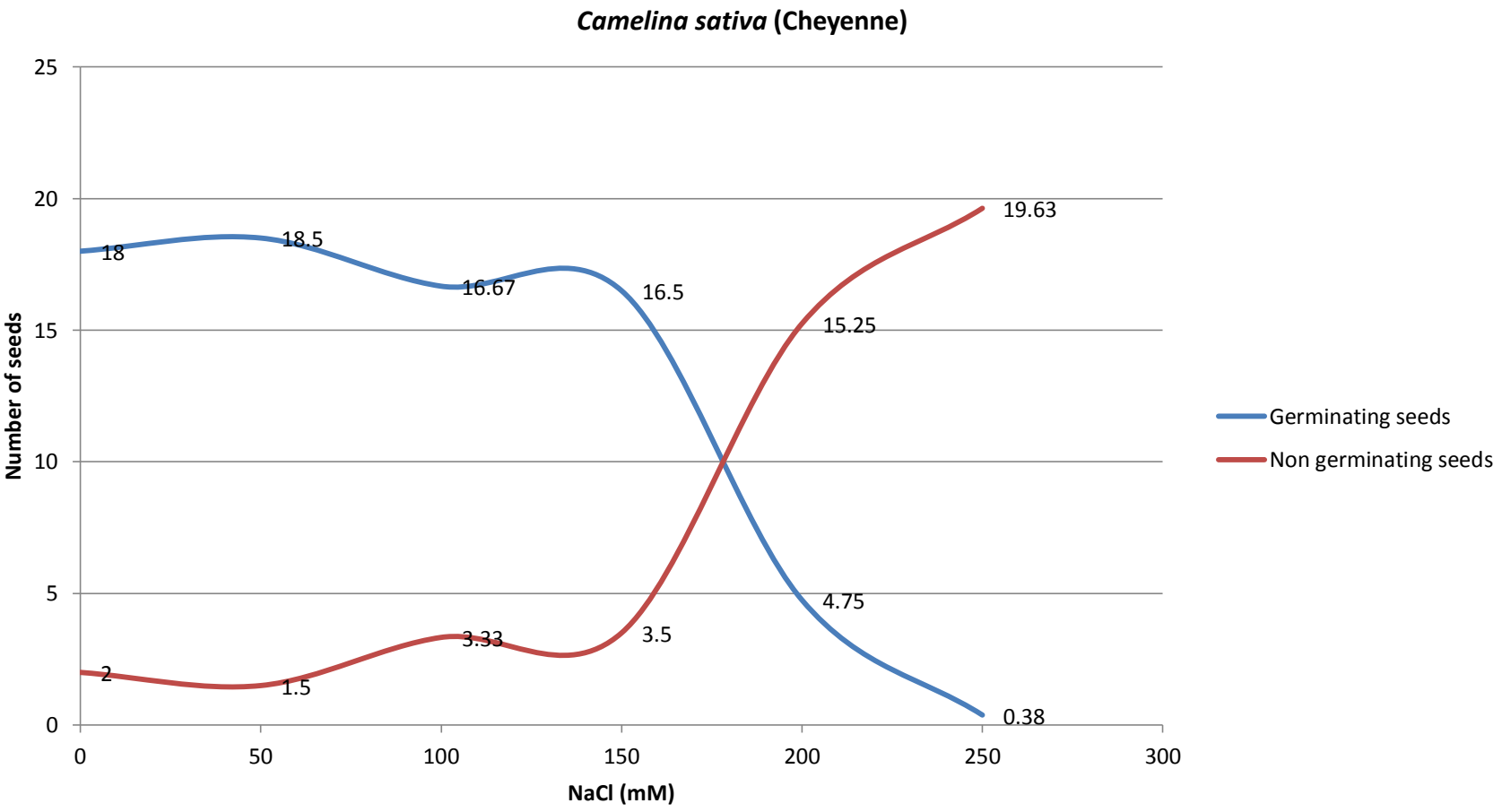
After 2 days



After 10 days

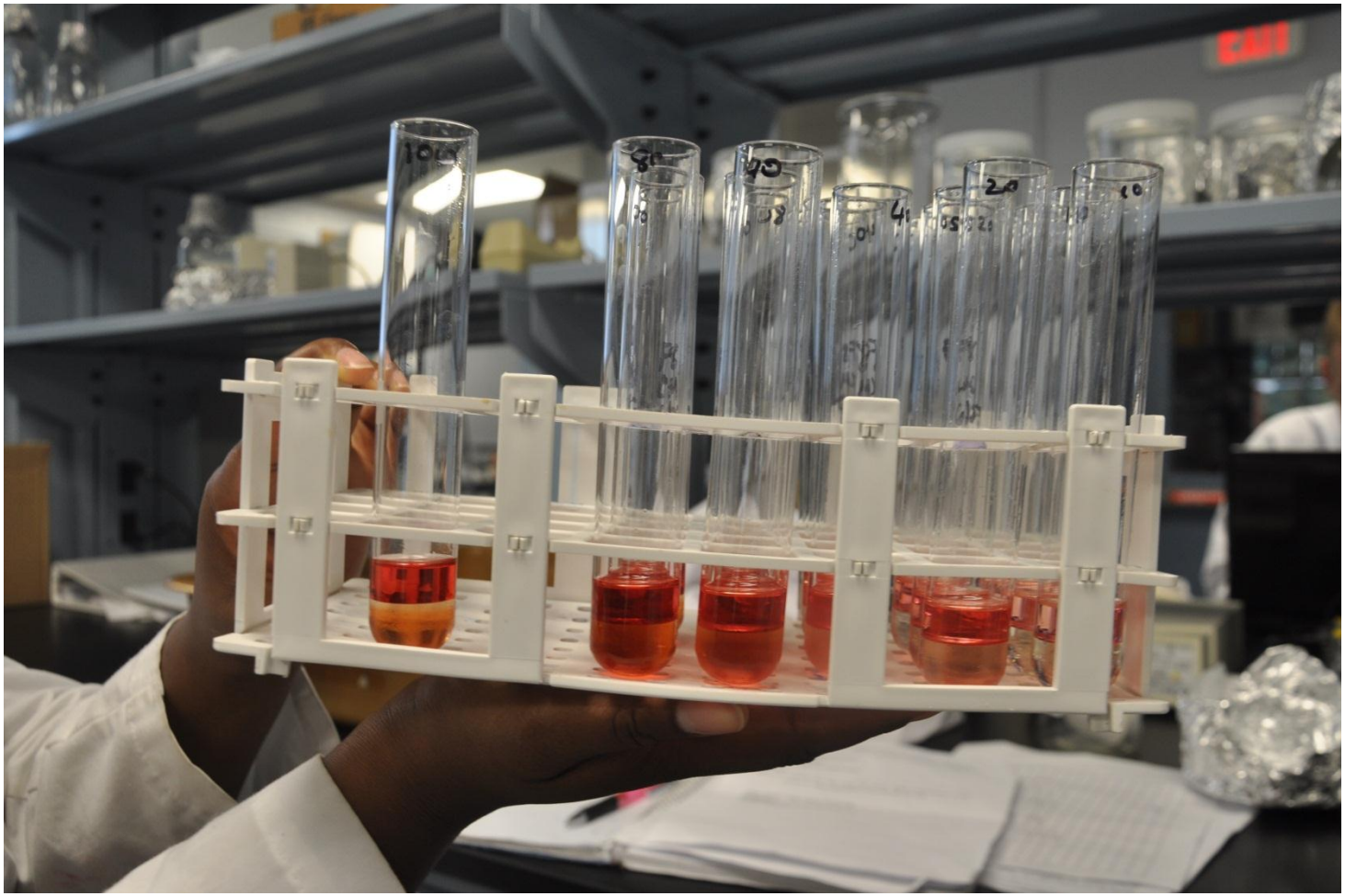


Germination of cv. Cheyenne

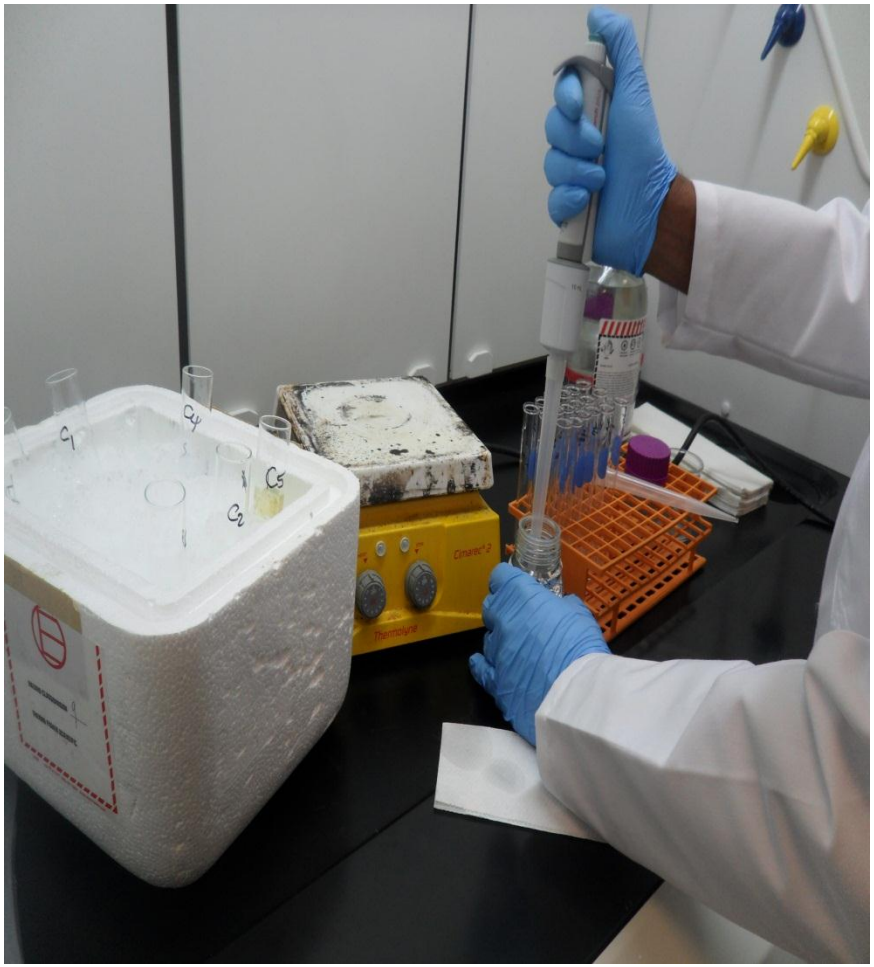


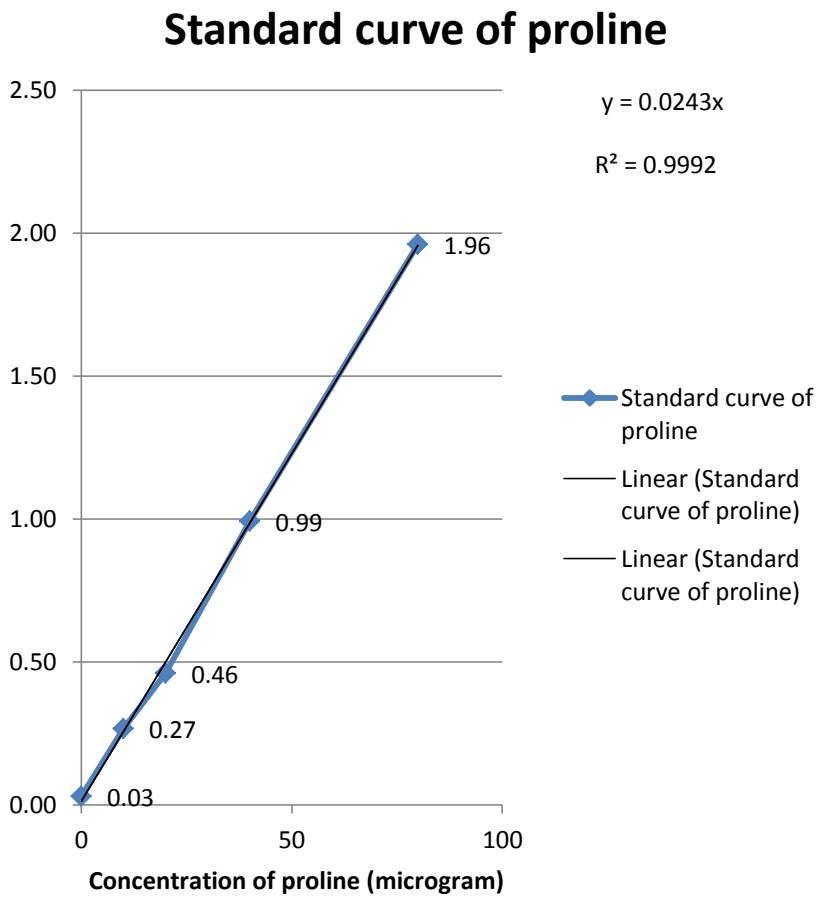
Standard curve graph procedure

- Take 20 mg/ml of proline, homogenize in 5 ml of 3% sulphosalycylic acid, and filter.
- Take 2 ml of proline in test tubes, add 2 ml of glacial acetic acid, and 2 ml of ninhydrin reagent.
- Heat reaction in a boiling water at 100 degree Celsius for 1 hr. Brick red color will develop.
- After cooling add 4 ml of toluene and transfer to separate tubes.
- Set the spectrophotometer at an absorbance of 520nm.
- Prepare standard curve of proline by taking 10 to 80 µg/ml concentration.

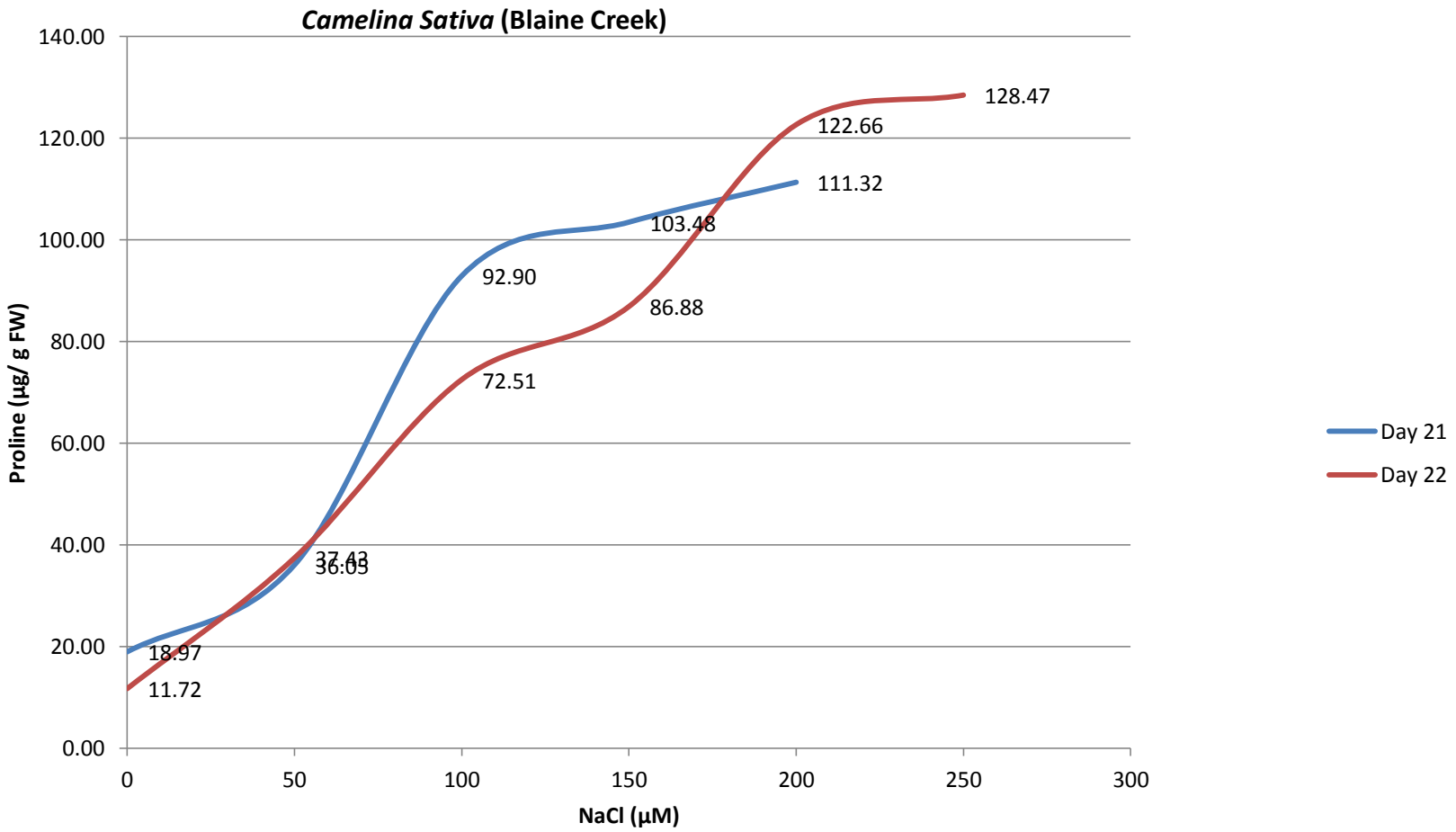


Standard graph for proline





Proline content of NaCl stressed C. sativa cv. Blaine Creek



Summary

- Seed germination and growth (Fresh weight) of Camelina were affected by NaCl salinity to different extends in different varieties
- Proline contents of the seedlings were increased by 50-250 mM salt to different extends
- Experiments are being repeated to validate this preliminary data