# Development of an *in vitro* cloning method for Basin big sagebrush to support genome sequencing and GXE research

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GEM3 Genes by Environment Modeling · Mechanisms · Mapping

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## Background

Sagebrush (*Artemisia tridentate;* Asteraceae) is an iconic, essential part of the ecosystem in the Intermountain West, which covers about a third of North America. Sagebrush provides cover and forage for many species (i.e. sage grouse, pronghorn and mule deer), soil stability, and soil enrichment through hydraulic lift and leaf decomposition[1]. Due to the importance of sagebrush, there is much interest in preserving it on the landscape. However, as climate change proceeds and drought events become more frequent, sagebrush is facing an uncertain future.

To determine what genes may be underpinning sagebrush adaptive capacity to drought, and therefore assess its success in a changing climate, we aim at assembling a *de novo* genome for the diploid (2x=18) *A. tridentata* ssp. *tridentata* (Basin big sagebrush).

To achieve this goal we intend to establish and maintain a cloning and tissue propagation program, which will provide the large amount of tissue needed for genome sequencing (~300 g). Additionally, this clonal line of sagebrush, with a known reference genome, will also allow us to conduct GxE (genotype by environment) experiments to study genomic adaptations to drought.

An efficient protocol for micropropagation has yet to be determined for sagebrush plants. Although progress has been made in this field, previous methods of cloning sagebrush have resulted in low root formation on shoots derived from callus.[2]

## Research Goals

We aim to develop the first protocol to ultimately establish a clonal line for *A. tridentata* ssp. *tridentata* and describe this methodology to carry out long-term micropropagation techniques.
We intend to develop a technique that will:

- Minimize potential mutation effects on clonal lines (i.e. somaclonal variation)
- Provide fast growth to ensure biomass production within the next year (2020) and
- Be financially viable

For this purpose, we are developing an *in vitro* propagation procedure via shoot tip culture. This approach is commonly used for clonal propagation and maintenance of genetic uniformity in horticultural crops.

## Materials and Methods

- About 300 seeds from one mother plant (IDT1-10) were surfaced-sterilized and germinated in tissue culture.
- Medium contained ½ strength Murashige and Skoog (MS), Gamborg vitamins, 1% sucrose, 1mL/L PPM (plant preservative mixture) and 0.3% phytagel.
- Seeds had a 67% germination rate.
- Seedlings were grown in culture for ~5 months before beginning experiments.

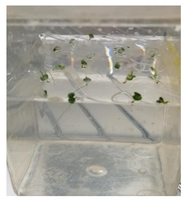
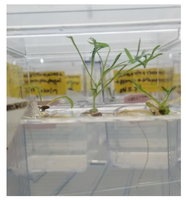


Figure1: From left to right 9-day, 30-day, and 60-day old seedlings.

## Experiment 1 – Does the media matter in initiating adventitious roots?

### Materials and Methods

Based on previous research with Wyoming big sagebrush[2], we investigated the formation of adventitious roots in two media: 1) the same MS medium used to germinate the seedlings and 2) a modified Fahräeus medium (i.e. one lacking nitrogen), which is commonly used to induce adventitious roots in other species[3].

- Twelve 12 x 12 cm Petri dishes were prepared, 2 containing the MS medium and 10 containing the modified Fahräeus medium.
- Twelve shoot tips were cut from each of 10 five-month old seedlings and one shoot tip per seedling was placed in a Petri dish.
- Petri plates were wrapped with Parafilm, placed at a 45° angle and incubated at room temperature (~23C).

### Results

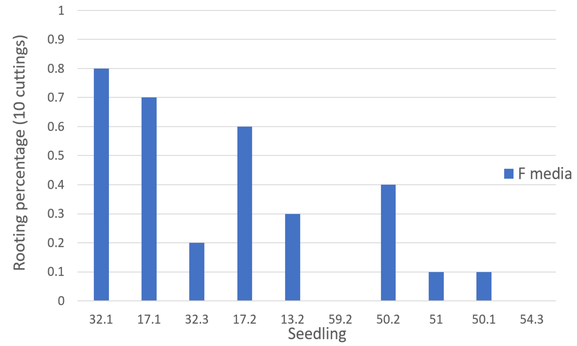


Figure 2: Rooting percentage per seedling in Fahräeus medium after two weeks in culture. Marked differences were observed between seedlings in their ability to form adventitious roots. Shoot explants from two seedlings did not form any roots, while explants from other seedlings reached rooting percentages of up to 80%.

Figure 3: Shoot tips in Fahräeus medium.

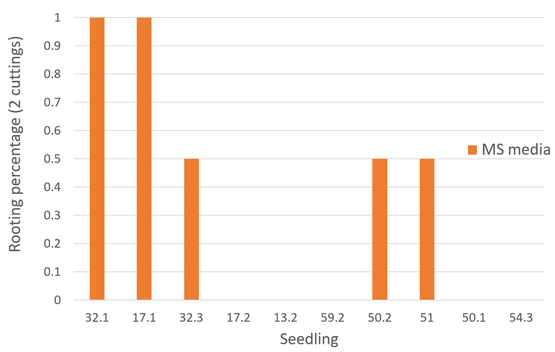


Figure 4: Rooting percentage per seedling in ½ MS medium after two weeks in culture. Even though the number of replications was minimal, the results in the MS medium show similar trends to those in the Fahräeus medium. The seedlings with the highest percent rooting in the Fahräeus medium also rooted in the MS medium, while those that did not form adventitious roots in the Fahräeus medium did not form roots in the MS medium.

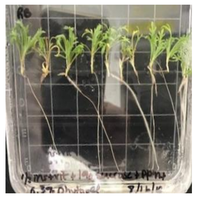

Figure 5: Shoot tips in ½ MS medium.

## Experiment 2 – Does the contact with the medium affect the formation of adventitious roots?

### Materials and Methods

In the previous experiment, the shoot tips were laid on the medium with their cut ends exposed to air. We hypothesized that better contact with the medium would improve rooting by reducing dehydration and allowing better exchange of nutrients[4]. To test this notion, rooting was assessed in cuttings where the basal ends of shoots tips were inserted into the culture medium.

- Ten shoot tips from each of 5 seedlings were placed in Magenta® boxes containing 100 mL ½ MS medium
- Explants were incubated at 23C in a Percival® tissue culture chamber.

### Results

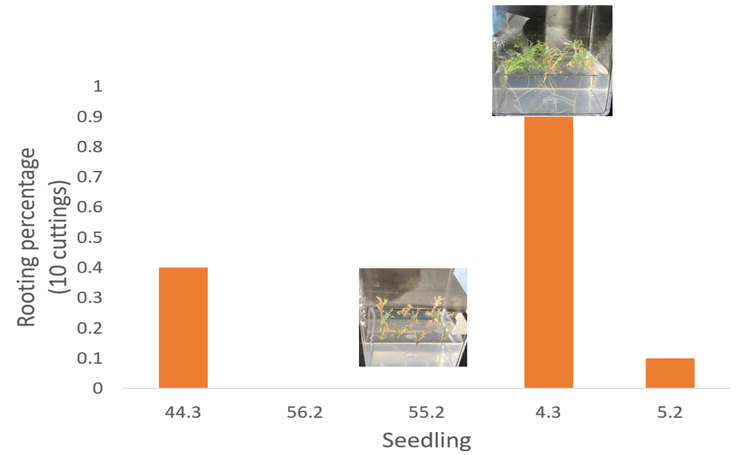


Figure 6: Rooting percentage per seedling in Magenta® boxes with ½ MS media after two weeks in culture. The results were similar to those observed in Experiment 1, suggesting that insertion in the medium does not affect rooting. The main factor affecting rooting appears to be the individual seedling from where the explants originated.

## Conclusions and Future Work

A method was developed to clone sagebrush seedlings. However, more research is needed to understand why shoot explants from some seedlings readily root with the method used, while others do not. Current experiments are aimed at investigating this question and overall optimize the cloning procedure.

## Literature Cited

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