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Development of an *in vitro* Method of Propagation for *Artemisia Tridentata* subsp. *tridentata* to Support Genome Sequencing and Genotype-by-Environment Research

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Abstract

Basin big sagebrush (*Artemisia tridentata* subsp. *tridentata*) is a keystone species of the sagebrush steppe, a widespread ecosystem of western North America threatened by climate change. The study's goal was to develop an *in vitro* method of propagation for this taxon to support genome sequencing and genotype-by-environment research on drought tolerance. Such research may ultimately facilitate the reintroduction of big sagebrush in degraded habitats. Effects of IBA and NAA on rooting of shoot tips were tested on 45 individuals and 15 shoot tips per individual. Growth regulator and individual-seedling effects on percent rooting and roots per shoot tip were evaluated using statistical and clustering analyses. Furthermore, rooted shoot tips were transferred into new media to ascertain their continued growth *in vitro*. Results suggest that *A. tridentata* is an outbred species, as shown by individuals' effect on rooting and growth. IBA addition was the most effective method for promoting adventitious rooting, especially in top-performing individuals. These individuals also have high survival and growth rates upon transferring to new media, making them suitable candidates for generating biomass for genome sequencing and producing clones for genotype-by-environment research.

Keywords

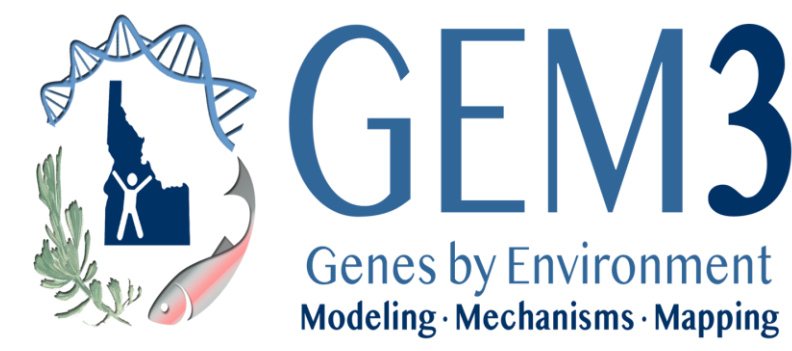
biological and life sciences, Genome 2 Phenome

Comments

This research is part of the Genome 2 Phenome project.

Rachael Barrons is also affiliated with Simplot Plant Sciences in Boise, Idaho.

Development of an *in vitro* method of propagation for *Artemisia tridentata* subsp. *tridentata* to support genome sequencing and genotype-by-environment research



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INTRODUCTION

- Basin big sagebrush (*Artemisia tridentata* subsp. *tridentata*) is a keystone species of the sagebrush steppe ecosystem and is threatened by climate change.
- An *in vitro* method of propagation will provide a route to produce a large amount of tissue for genomic sequencing.
- A clonal line of sagebrush, with a known reference genome, will allow for genotype-by-environment experiments to study genomic adaptations to drought.
- Ultimately this research could facilitate the reintroduction of big sagebrush in degraded habitats.

METHODS

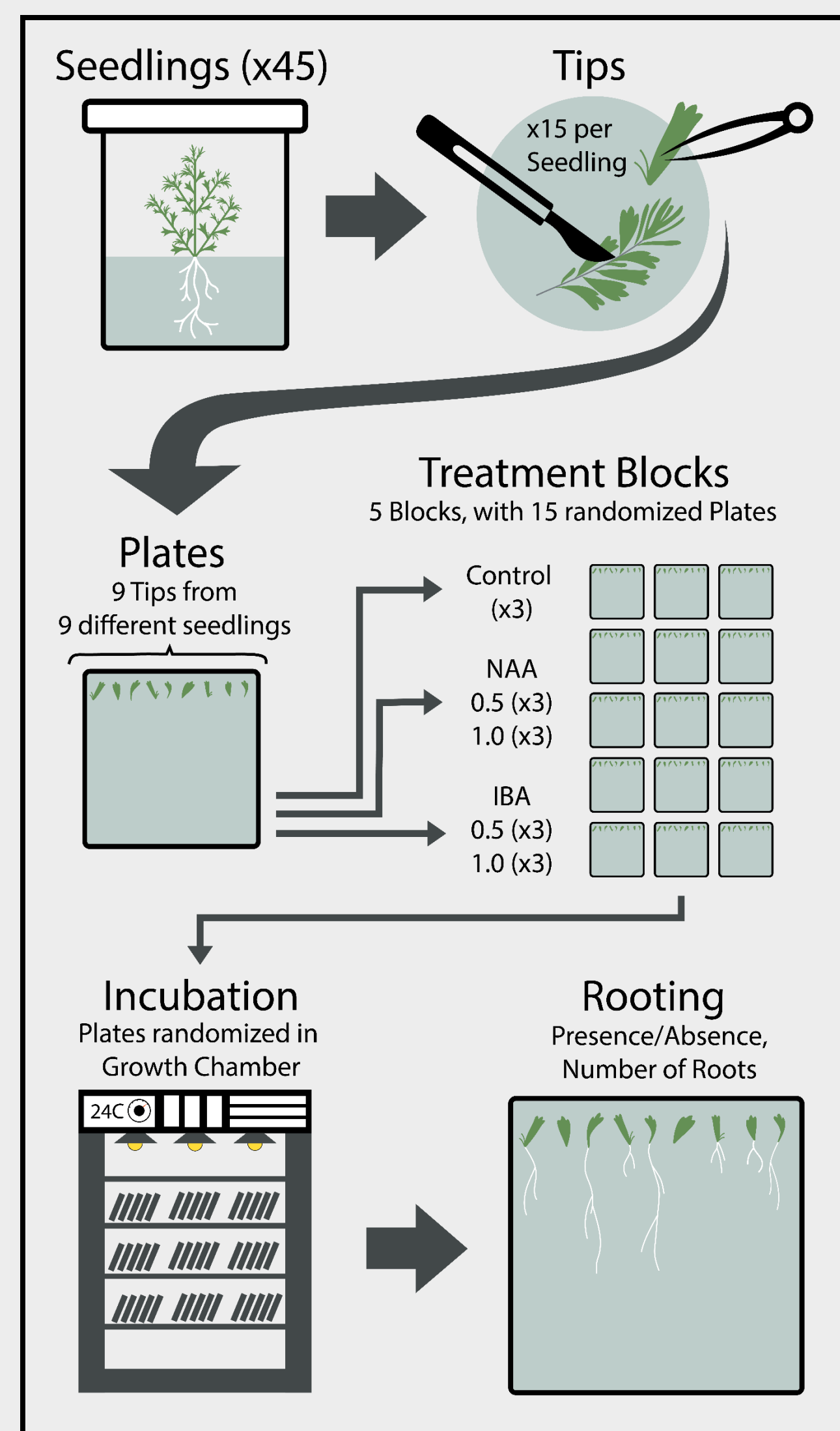


Figure 1: Schematic showing methods and experimental design for propagating clones of sagebrush.

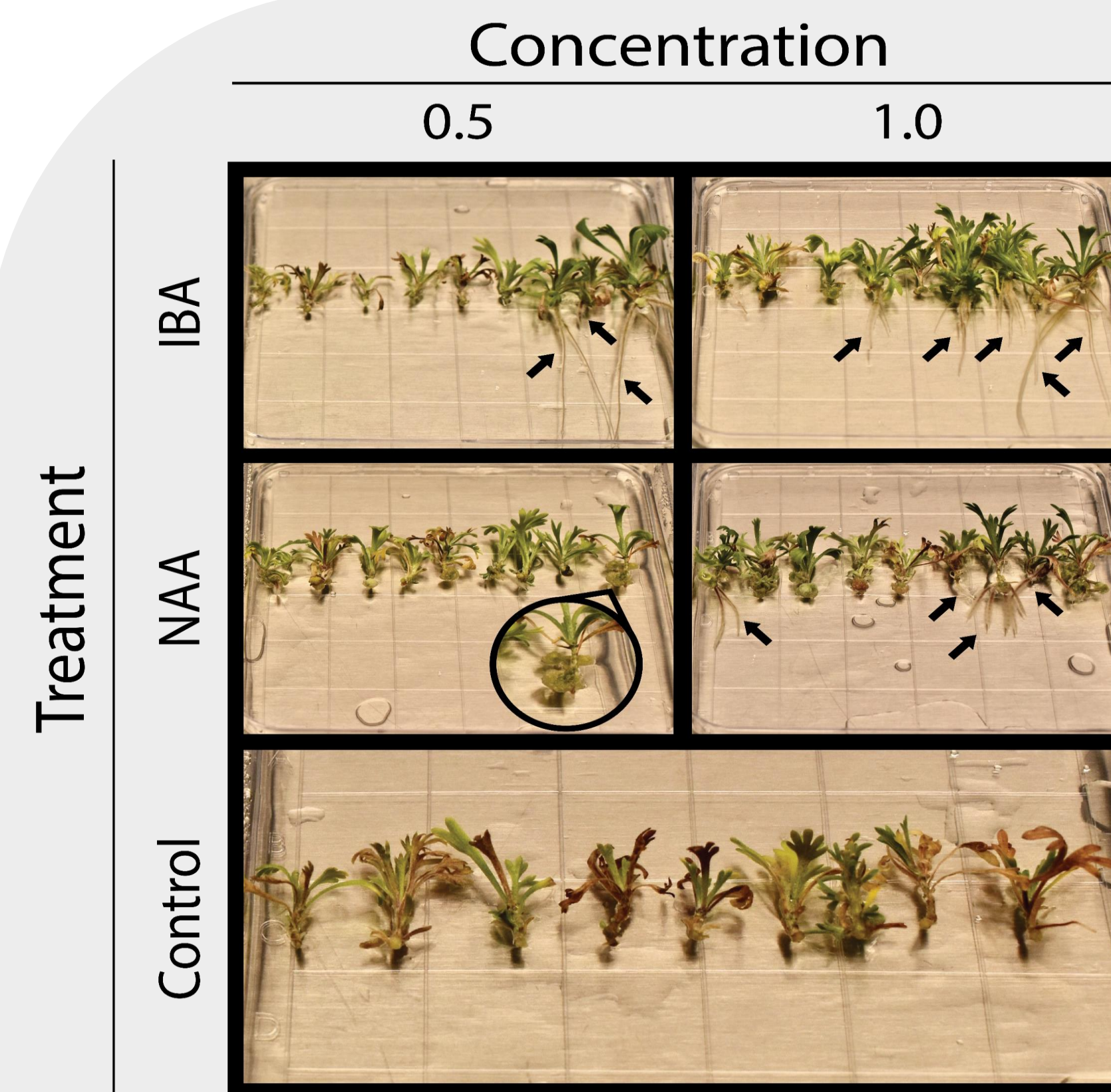


Figure 2. Representative shoot tips showing both roots (arrows) and callus (magnified zone) in treatments after 15 days.

- Clustering analysis based on number of roots per shoot tip revealed three clusters
 - Blue → high rooting capacity
 - Pink → reduced rooting capacity
 - Black → limited rooting capacity

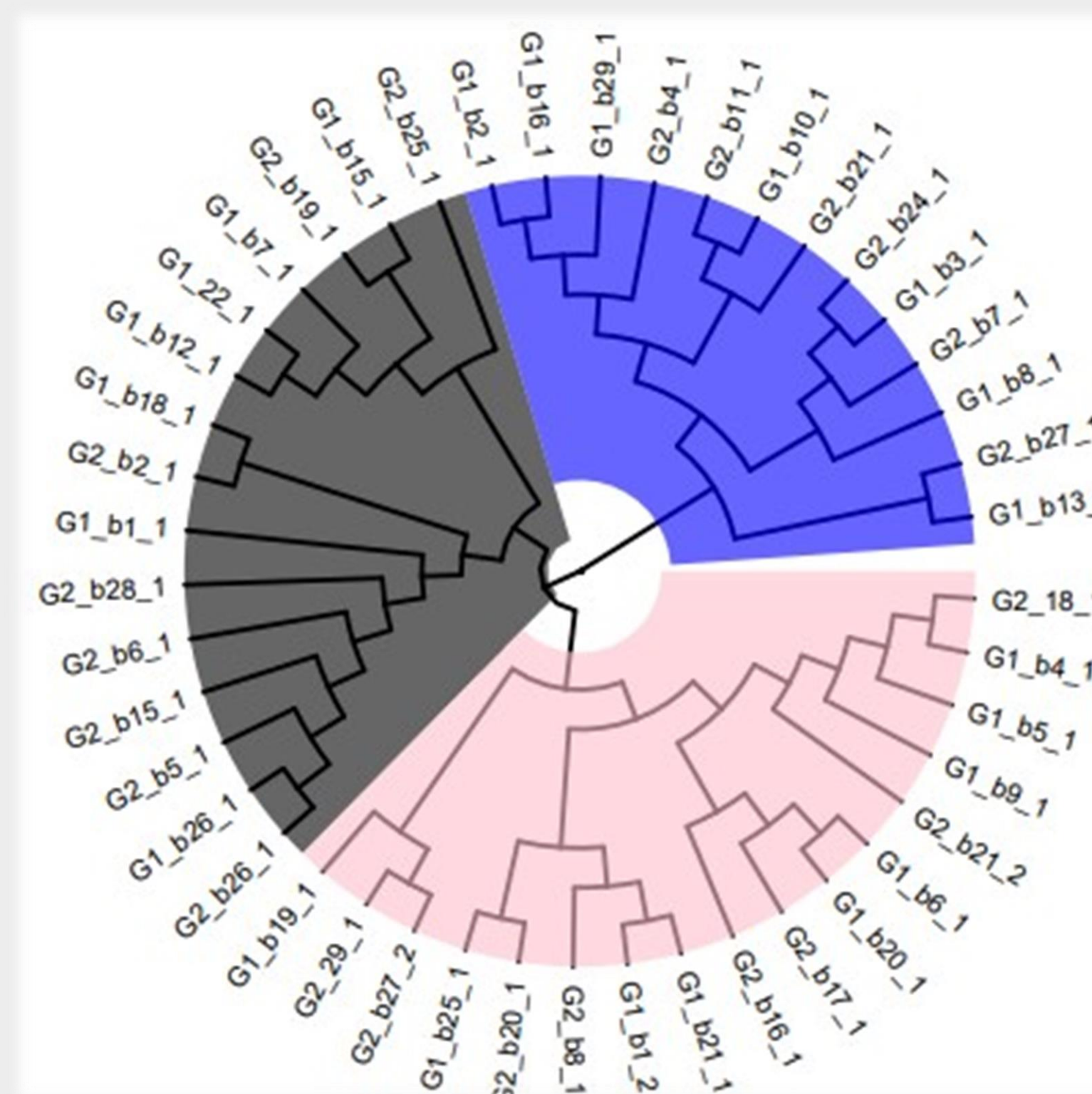


Figure 3. Clustering analysis based on root data. Clusters are represented by shaded polygons

RESULTS

- IBA 1.0 mg/L addition was the most effective auxin to initiate adventitious rooting (60% rooting response).
- There was a strong individual effect on rooting percentage.

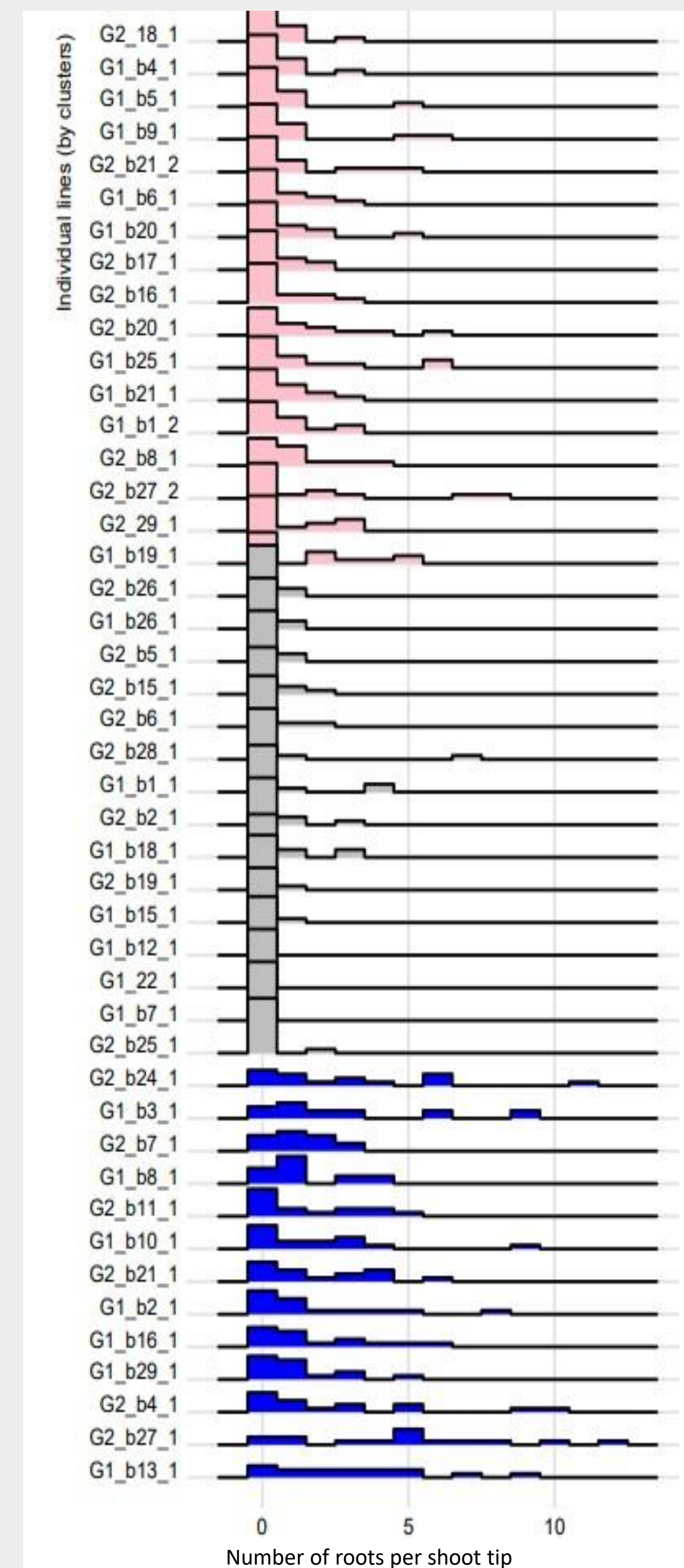


Figure 4. Ridgeline plot comparing root formation in shoot tips of *Artemisia tridentata* subsp. *tridentata*

RESULTS

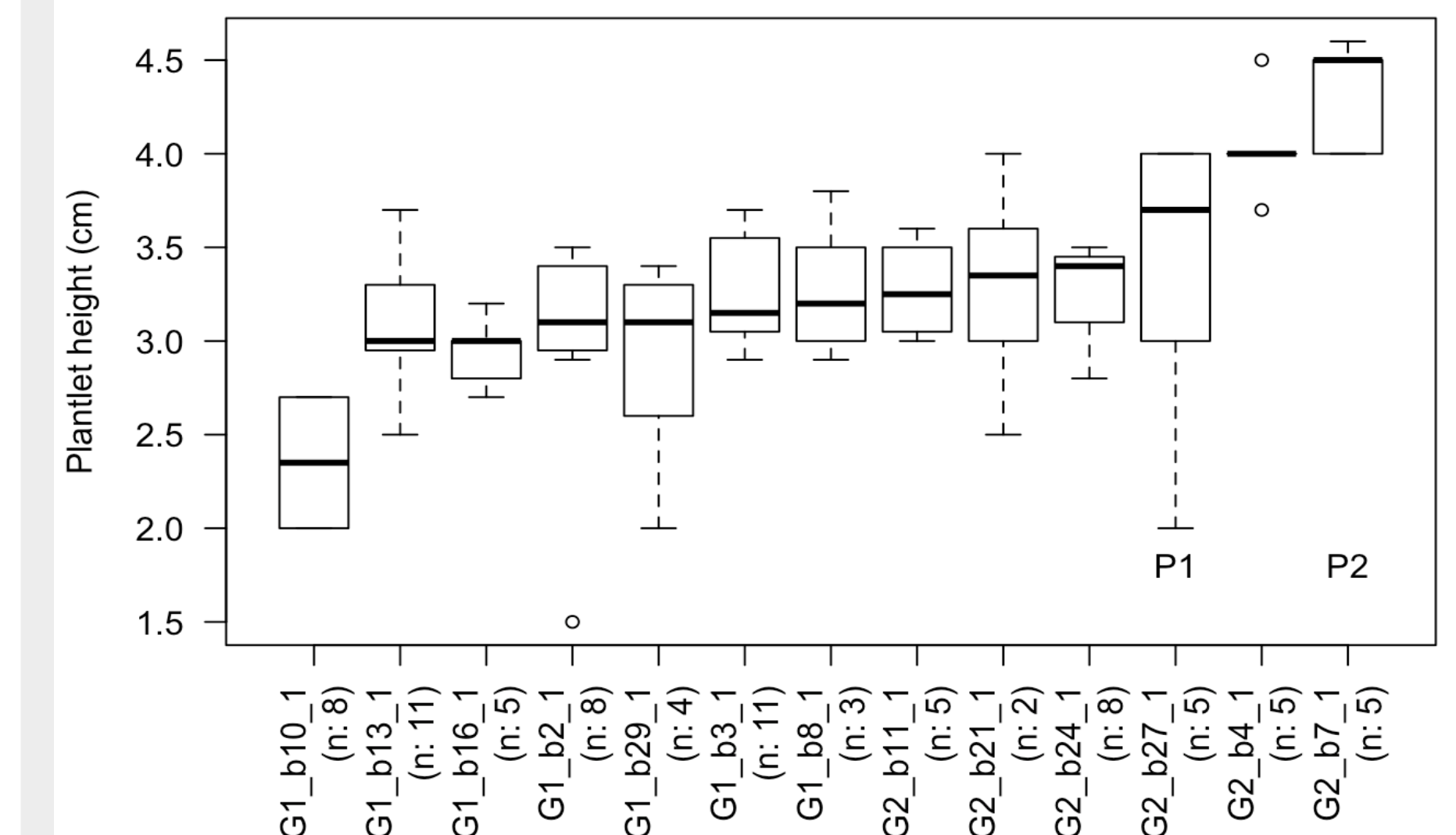


Figure 5. Boxplot showing plantlets heights after five weeks of culture for individuals belonging to the blue rooting cluster of *Artemisia tridentata* subsp. *tridentata*. The n indicates the number of plantlets cultured for each individual. The “P” indicates the top performers by the statistical analyses..

- Two individual lines were identified as being significantly better at rooting and growth (>=20%) G2_b27_1 and G2_b7_1

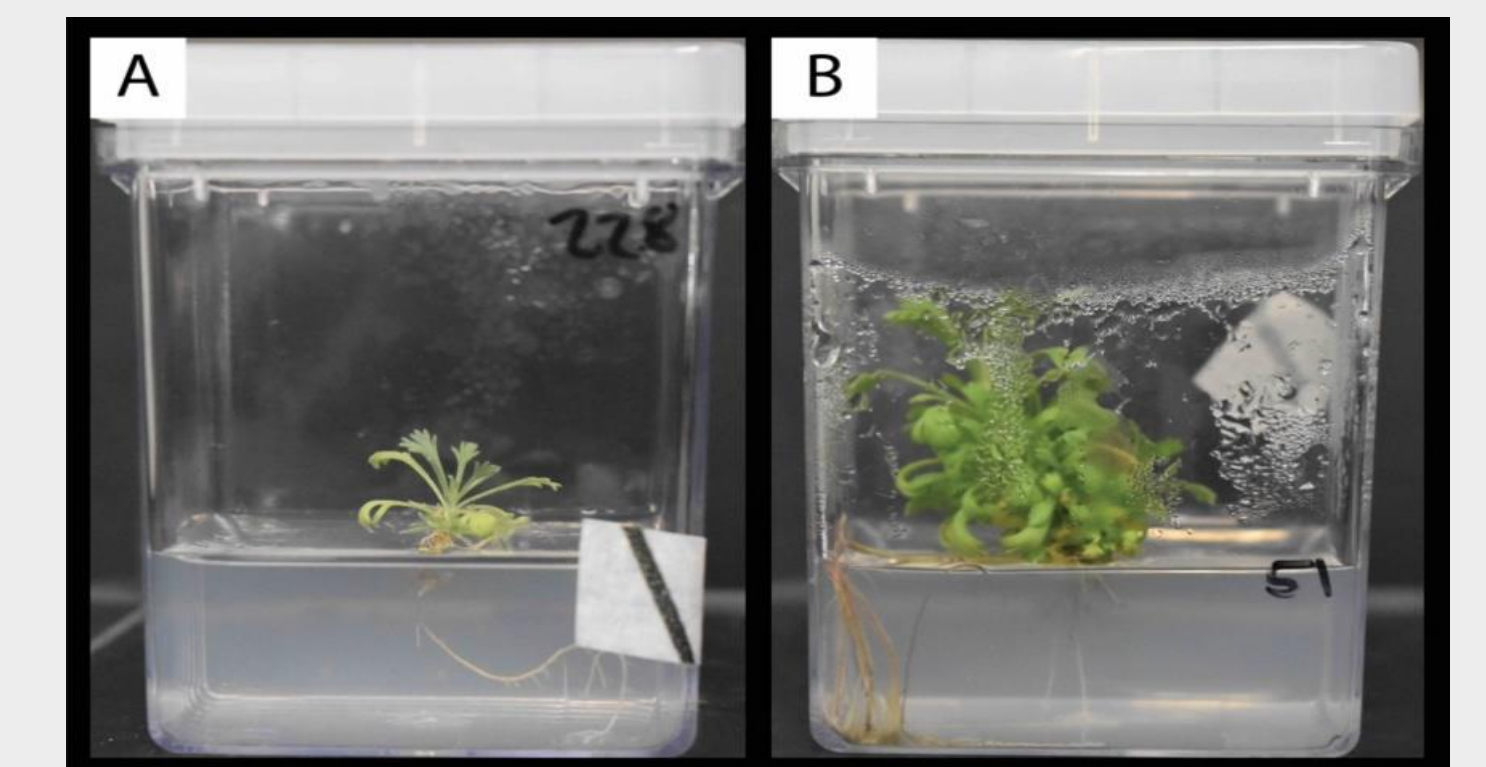


Figure 6. Plantlets from different individual parents after 5 weeks of growth.

NEXT STEPS

- Top performers identified in this study will be propagated to create a **reference genome** for *Artemisia tridentata* subsp. *tridentata*.
- ~800 plantlets are required to reach ~120g of tissue needed for genomic sequencing.
- Plantlets with a known genome could then be transferred to soil and ultimately into the field for genotype-by-environment research.