Comparing the effects of cytokinin source and concentration on shoot growth in a micropropagation system of UCB-1 interspecific Pistacia rootstock

By

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Abstract

Protocols for the micropropagation of pistachio rootstock are not well established, and little is known about the effect of different plant growth regulators (PGRs) on micropropagation systems of the UCB-1 pistachio rootstock. Two cytokinins, 6-benzylaminopurine (BAP) and meta-Topolin (*m*T), were tested at three different concentrations (1uM, 5uM, 10uM) to germinate, multiply, and root UCB-1 pistachio seedlings *in vitro.* UCB-1 seedlings germinated at a rate of 76% in clean culture after being split with bypass pruning shears and a 3hr decontamination treatment in 3000ppm sodium dichloroisocyanurate (NaDCC) solution. Data were collected on cumulative shoot length, weight, and branching of single-shoot descent lines. The 10uM concentration of both tested cytokinins provided the greatest amount of shoot material at the end of three transfer cycles, though no significant difference ($P < 0.05$) in shoot growth was found between the two cytokinins at that concentration. Mean rooting performance was significantly ($P < 0.001$) higher for shoots multiplied with BAP (87.2%) as the source of cytokinin compared to *m*T (57.9%).

Acknowledgements

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Particular thanks is due to Caitlin S. Beach (University of Michigan) for her substantial help with the statistical analysis and for providing R scripts for this project.

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Abbreviated terms

K-IBA- Indole-3 butyric acid, Potassium salt
*m*T- meta-Topolin
BAP- 6-benzylaminopurine
PGR- Plant Growth Regulator
DI water- deionized water
NaDCC- sodium dichloroisocyanurate
FeEDDHA- iron(III)-sodium salt
FPS- UC Davis Foundation Plant Services

Introduction

Pistachios grown in California rely on rootstock for continued nut production due to pests and diseases such as *Verticillium dahliae, Phytophtora* spp*.,* and nematodes that would otherwise degrade *Pistacia vera* trees grown on their own roots. Previous generations of pistachio growers in California relied solely on seedling rootstocks due to the difficulty of rooting *Pistacia* spp. Development of seedling rootstocks eventually led to UCB-1 (*P. atlantica* x *P. integerrima*) and PGII (*P. integerrima* x *P. atlantica*) hybrids which combine the Verticillium tolerance of *P. integerrima* with the cold and salt tolerance of *P. atlantica* (Ferguson et al 2016).

Seedling rootstocks inherently have high genetic variability, and improvements to micropropagation techniques recently enabled clonal propagation of superior individuals. Current clonal pistachio rootstock varieties available are limited to either a handful of UCB-1 selections or the lone selection from PGII, "Platinum." The California Pistachio Research Board (2021) has funded research and testing of new rootstock hybrid families which may be able to provide new biotic tolerances or increased resilience against biotic pests; however, new rootstock hybrids will take considerable time to evaluate and gain the widespread grower confidence held by UCB-1. In the interim, UCB-1 is currently the only commercially available seedling rootstock, and therefore the only population for generating new clonal selections in California. Developing protocols that can be broadly applied to new UCB-1 clonal lines will require testing various single-shoot descent lines of specific genotypes *in vitro*. The introduction of new, clean, seedling material into culture would allow for faster testing of multiple new clones; however,

there is no published protocol for germinating UCB-1 seed *in vitro.* An ideal in vitro germination protocol for UCB-1 seeds would provide clean germinated plants that are direct sown *in vitro.* This would skip much of normal seed propagation process and allow for new material to be introduced into culture while skipping greenhouse germination, multiple repotting cycles, and final introduction into clean culture.

The difficulty of developing new clonal pistachio rootstock selections is compounded by *Pistacia spp*. being notoriously difficult to propagate from rooted cuttings. As such, a limited number of publications have demonstrated successful traditional greenhouse propagation methods (Almehdi and Parfitt 2002, Barazi 1982) with micropropagation continuing to show the recalcitrant nature of pistachios. *In vitro* cultures of *Pistacia* spp. with high multiplication rates often require relatively high concentrations of cytokinin for multiplication (Benmahioul 2017, Abousalim 1991), and subsequently high concentrations of auxin for maximum rooting (Tilkat 2009). Previous work done by Benmahioul et al. (2011) compared seedling *P. vera* on media containing one of three different cytokinins, including either meta-Topolin (*m*T) or 6-benzylaminopurine (BAP), and showed that various seedling *P. vera* derived clonal lines produced more usable shoots and a measurably higher percentage of rooted plantlets when cultured on media containing *m*T as the principal Plant Growth Regulator (PGR) compared to those cultured with BAP.

There is a large cost difference between *m*T and BAP, with BAP often being 50-100 times less expensive than *m*T at common chemical suppliers. Therefore, UCB-1 shoot growth metrics on medium with *m*T compared to BAP may not be the only factor for deciding the efficacy of introducing *m*T to a culture system. Based on previous work by Benmahioul et al. (2011), it is expected that pistachio shoots cultured with *m*T would perform at least as well as BAP in quantitative shoot multiplication measures (branching, fresh weight gain, total shoot length). Use of the cytokinin *m*T is also expected to improve subsequent rooting performance.

Materials and Methods

Germination

To establish a germination protocol, UCB-1 seeds provided by UC Davis Foundation Plant Services (FPS) were bisected along the short axis with bypass pruning shears or not cut at all. Both sets of seeds were placed into a decontamination solution of 3000ppm Sodium Dichloroisocyanurate (NaDCC) plus 1 drop of Tween® 20 per liter, then circulated on an orbital shaker in sealed polypropylene containers for 3 hours. After 3 hours, the containers of seeds were transported to a laminar flow hood where the decontamination solution was decanted and the seeds were placed without rinsing directly onto Pistachio Media consisting of the labeled rate of commercial DKW basal salts and vitamins (Product ID D2470, Phytotech Labs, Lenexa, Kansas, USA) as described by Driver and Kuniyuki (1984) and McGranahan (1987) modified with 80uM iron(III)-sodium salt (FeEDDHA), 30g/L sucrose, pH adjusted to 5.7 with 6g/L Micropropagation Grade Agar (Product ID A111, Phytotech Labs). For germination studies 5uM BAP was used as the PGR and seeds were assessed for germination after 4 weeks. Successful germination was defined as a seedling that produced shoot tips greater than 1cm, as measured from the cotyledons, after 4 weeks.

PGR Source and Applied Concentration Comparisons

For comparing the efficacy of different concentrations and sources of cytokinins, the Pistachio Media was made as described above. A 2x3 factorial design was generated with 1, 5, and 10uM BAP or *m*T. Twenty UCB-1 seeds per treatment were placed into their respective concentration of cytokinin for germination for a total of 120 seeds per run with two runs performed. After 4 weeks, one single 1cm tall shoot was excised above the cotyledons from each germinated seedling to exclude the cotyledonary buds. Each shoot line was repropagated every 4 weeks to produce as many 1cm tall shoots as possible while all branches were excised. To qualify as a distinct “branch”, a branch had to extend greater than

0.5 cm away from the main stem. At every repropagation, total shoot length, total fresh weight, and total branching of each line was recorded. Data presented are from the third repropagation cycle.

Rooting

After concluding the third transfer and taking data, only lines from the highest concentrations of PGR (10uM *m*T and 10uM BAP) were maintained. Shoots from maintained lines were subsequently placed into an established rooting protocol for UCB-1. This rooting protocol was based on previously published work with walnut (*Juglans* spp.; Hackett et al. 2009, Leslie and McGranahan 2009, Licea-Moreno et al. 2015). Shoots were induced in full strength Pistachio Media with 50uM Indole-3-Butyric acid, Potassium salt (K-IBA) for 1 week in darkness. After a 7-day induction period, shoots were then transferred to a Root Expression Medium consisting of ½ the labeled rate of a commercial DKW Basal Media with Vitamins (Product ID D2470, Phytotech Labs) amended with 80uM FeEDDHA, 30g/L sucrose, pH adjusted to 5.7 and 6g/L Micropropagation Grade Agar (Product ID A111, Phytotech Labs) with no PGR. After being placed in Root Expression media, containers with shoots were placed under white LED tube lighting with 16 hours of light and 8 hours of darkness. Rooting percentages were collected 3 weeks after shoots were placed on Root Expression Media and placed under the tube lighting.

Statistical Analysis

Data collected for this study were compiled and analyzed using R (R Core Team, 2018). Analysis of Variance (ANOVA) tables were produced for each measured response (branching, length, fresh weight, rooting) following a linear model (Response~PGR:Concentration+Run+Run:Rate:PGR) to determine the most likely sources of variation in the data. Estimated Marginal Means were contrasted using the emmeans package (Length et al. 2021) at the confidence level of 0.95 to assess significance, and visualizations were generated using the ggplot2 package (Wickham et al., 2021).

Results and Discussion

Germination

As the data in Table 1 shows, merely surface decontaminating intact seeds for 3h in 3000ppm NaDCC was insufficient to allow proper imbibition. Intact seeds were observed for an additional three weeks in semi-solid media after data collection and no additional seeds germinated. Cutting UCB-1 seeds (Fig. 3) is simple to perform without damaging the embryo due to the seed anatomy of *P. atlantica* (Fig. 4). Upon first inspection, UCB-1 seeds appear to be bilaterally symmetrical which, if true, would make it difficult to determine the side in which the embryo resided. However, key external features of the endocarp revealed the position of the embryo without needing to first open the shell. As shown in Figure 5, the hilum attachment is not central but skewed toward the embryo side in addition to the suture along the embryo side of the endocarp being thicker and having a puckered appearance.

PGR Source and Applied Concentration Comparison

Several factors must be considered when optimizing any tissue culture program. The type and concentration of cytokinin has a demonstrable effect on root expression *in vitro*; however, this study did not follow plant material to greenhouse survival, which is a critical step for any plant being used as a rootstock. There was little discernible difference between the cytokinins BAP and *m*T at the three tested concentrations (1uM, 5uM, and 10uM) across all three metrics (total shoot length, total branches, and fresh weight) at the $P < 0.05$ level of significance (Table 2). As demonstrated by the representative samples shown in Figure 6, low concentrations of cytokinins were observed to have poor shoot growth and little to no branching. Also shown in Figure 6, Total Shoot Length did not correspond to the plantlets with the tallest single shoot. As such, the tube shown for 5uM *m*T may have one single tall shoot but would have a lower value for total shoot length due to it having far fewer branches. With respect to weight, length, or branching quantity, a new UCB-1 single-shoot descent line could be expected to have similar performance at any given concentration of PGR regardless of whether BAP or *m*T was used in the

Pistachio Media. The only discernible differences between cytokinin sources in this study were seen at the 5uM concentration. The 5uM concentration of *m*T was not significantly ($P < 0.05$) different from 10uM *m*T in total length, while 5uM BAP was not significantly ($P < 0.05$) different from either BAP or *m*T at the 1uM concentration for branching. With regards to total fresh weight, 5uM *m*T was not significantly ($P < 0.05$) different from either source at the 10uM concentration. Across all single-shoot lines and both runs of the experiment, both PGR sources produced similar amounts of shoot material at the 10uM concentration (Figs. 8, 9, 10). Based on the data collected, it appears that UCB-1 may have limited response to *m*T concentrations above 10uM, but further testing is necessary to confirm.

Rooting

Optimizing any tissue culture program requires consideration of possible inhibition of rooting from the carry-over effects of PGRs/cytokinins used during shoot multiplication. In this study, for example, an assumption that the highest concentration of cytokinin would show the greatest rooting inhibition led to the decision to use the highest yielding shoot material from the multiplication step (10uM from both cytokinins) for measuring rooting. An alternative approach could consider total plants yielded from a set number of shoots over a given period of time. Luxurious shoot growth is a tempting metric by which to measure successful micropropagation. However, if the increased concentration of cytokinin to gain such shoot growth causes a precipitous drop in rooting performance, it may be more optimal to produce fewer total shoots with higher rooting rates. Rooting performance improvements of UCB-1 cultured on *m*T were not shown by this study. UCB-1 cultures in this study grown on media containing *m*T yielded significantly fewer ($P < 0.01$) rooted plants than shoots grown on media containing BAP (Table 3). The difference in rooting performance between the two cytokinin sources is demonstrated by Figure 7, in which shoots previously cultured on media containing BAP are rooting noticeably better (87.2$\pm$10.1%)) than shoots previously cultured with *m*T (57.9$\pm$9.3%). The ANOVA showed that the previous PGR used in the multiplication step played a significant ($P >0.001$) role in the subsequent rooting of *in vitro* shoots.

These results conflict with those reported for *P. vera* by Benmahioul (2017). This result may suggest UCB-1 cultures are less sensitive to the differences between cytokinins for shoot growth metrics compared to *P. vera* cultures, or it may be that the sample sizes in this study were insufficient to overcome confounding effects from genetic diversity of the UCB-1 seedlings. With the knowledge that the hard endocarp of UCB-1 seeds is the major barrier to germination *in vitro*, subsequent studies may be able to germinate seedlings in clean culture on a PGR-free medium and divide segments amongst the various cytokinins and concentrations. More direct comparisons would then be able to be made about those specific lines and their behaviors under the influence of different PGRs at different concentrations.

Conclusions

UCB-1 seeds required more than simple surface decontamination treatments to overcome the physical barrier to imbibition. UCB-1 seeds, due to the position of the embryo within the shell, survive being cut with bypass pruning shears and subsequent surface decontamination for 3h on a solution containing 3000ppm NaDCC.

Shoot branching, cumulative shoot length, and fresh weight were highest in UCB-1 single-shoot descent lines cultured with either 10uM BAP or *m*T. Therefore, no statement can be made about preferences for either *m*T or BA regarding shoot multiplication of UCB-1 single-shoot descent lines at the 10uM concentration. However, seedling-derived single-shoot lines multiplied on media containing 10uM *m*T as the cytokinin had significantly ($P > 0.001$) poorer rooting performance compared to those multiplied on media containing 10uM BA. This result differs from results on *P. vera* reported by Benmahioul et all (2011), however, many untested factors may contribute to the difference in results.

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Appendix 1

UCB-1 Seed Preparation and Germination Trials.

The requirement for clean seed for this project required new protocols to be developed beyond the standard protocols from FPS for greenhouse seed propagation. Initial work with *Pistacia* spp. showed that pistachios could be germinated in PGR-free medium without stratification if the endocarp was removed. Direct sowing of surface-decontaminated seeds with intact unsplit shells yielded no germination *in vitro*. These initial tests demonstrated that the most important inhibition of germination for UCB-1 seeds was the physical barrier to imbibition. Therefore, other methods of germination needed to be developed to establish a labor efficient method to generate seedlings *in vitro*. Several attempted methods to germinate UCB-1 seeds *in vitro* failed to overcome the physical barrier to imbibition of the pistachio endocarp.

Barriers to Imbibition

The requirement to germinate UCB-1 seeds *in vitro* required the use of a surface decontamination solution of some kind, as well as an appropriate imbibition of the seed to allow germination. Surface decontamination testing demonstrated that both sodium hypochlorite bleach and ethanol treated materials demonstrated low levels of contamination, as expected, but damaged exposed tissues soaked for 1hr or more. Therefore, the use of either bleach or ethanol for surface decontamination necessitated the added step of imbibing seeds separately with sterilized deionized (DI) water. Testing with NaDCC demonstrated that field material, *in vitro* shoot material, or greenhouse material could be sufficiently surface decontaminated by a 3hr soak in a 3000ppm NaDCC solution. The comparatively short duration surface decontamination times required with ethanol and bleach to avoid potential tissue damage were insufficient to adequately imbibe water, and the 3hr soak in 3000ppm NaDCC also adequate decontamination but resulted in no germination. Soaking of *Prunus dulcis* (almond) seeds for FPS provided adequate germination, low contamination rates, and softened seed coats after 24hr with a solution of 100ppm NaDCC with no perceptible damage to germinating tissues. Based on these results,

it was theorized that soaking UCB-1 seed for 24, 48, or 96 hours in 100ppm NaDCC could provide sufficient imbibition to facilitate germination. After each soak duration, UCB-1 seeds were placed on semi-solid Pistachio Media and observed for germination and contamination. Contamination rates were less than 1%, across the approximately 200 total UCB-1 seeds placed on Pistachio Media. However, germination rates were unacceptably poor with the highest rate observed in any container being 5% and the few seeds to germinate only did so slowly and sporadically (Fig. 1). Due to the lack of success with the previously described methods, it was theorized that the hard, tightly sealed endocarp was likely inhibiting imbibition and other scarification methods were attempted.

Chemical Scarification

With particular attention paid to limiting the number of steps to any protocol, chemical scarification attempts were pursued. It was theorized that treatment with strong acid to the endocarp of UCB-1 seed would also surface sterilize any treated seed. Multiple soak durations in various concentrations of sulfuric acid (H_2SO_4) were attempted with the best results coming from long duration soaks in high concentrations, thus softening the hard endocarp of UCB-1 without damaging the seed inside but allowing a scalpel blade to penetrate. The final chemical scarification procedure (Fig. 2) floated seeds in as small a volume of 36N H_2SO_4 as could be used for 7 hours without stirring, followed by three 15-minute rinses in sterile DI water, softened the endocarp sufficiently to allow a scalpel blade to penetrate it without breaking. This method damaged seeds with slight imperfections to the endocarp and proved time inefficient due to the initial 7-hour soak. Sterile rinses were extremely caustic due to the high concentration of H_2SO_4, which also added a considerable amount of additional time for neutralization and clean up.

Physical Scarification

Due to the hardness of *Pistacia* spp. shells, some methods of physical scarification proved more difficult than others. Due to the variability in size between seeds, locking pliers required constant adjustment

and non-locking pliers crushed a large proportion of embryos. A small bench vice used for opening the endocarps other drupes provided much greater control than pliers; however, this method appeared to be more suitable for unsplit *P. vera* shells as its larger seeds provide a more predictable trajectory. Using the bench vice produced small numbers of intact seeds which were subsequently surface decontaminated in NaDCC. Surface decontaminated seeds removed from the endocarp germinated, which provided support for the hypothesis that a major barrier to germination *in vitro* was the endocarp preventing imbibition. Bypass pruning shears commonly used in field work were later tested, and it was discovered they could cut through the hard *Pistacia* spp. endocarp without crushing the seed inside (Fig. 3). Using bypass pruning shears to cut through the hard endocarp allowed much of the tedious manual labor to be performed outside of sterile conditions, which could be subsequently surface decontaminated in NaDCC for germination.

Tables and Figures

Table 1. UCB-1 seed germination *in vitro* comparing decontaminated vs cut + decontaminated seed. UCB-1 seeds cut in half along the short axis with pruning shears were able to generate clean healthy seedlings *in vitro*. This is in sharp contrast to intact UCB-1 seeds which failed to germinate in this study.

Treatment	n	Contamination	Clean, Failed Germination	Clean Germination	Clean Germination (%)
Run1					
Cut	32	1	7	24	75
Intact	32	2	30	0	0
Run2					
Cut	30	0	7	23	77
Intact	28	0	28	0	0
Total					
Cut	62	1	14	47	76
Intact	60	2	58	0	0

Table 2. Comparison of averages among all genotypes across all treatments. For all three metrics, neither PGR is discernible at the 1uM or 10uM concentrations, although the 5uM *m*T produces more shoot material than 5uM BAP, the 10uM concentration of both PGRs produced the greatest quantity of shoot material. 5uM *m*T was not shown to be significantly different from the 10uM concentrations at P< 0.05 for fresh weight, but produced significantly less total shoot length and fewer branches than 10uM BAP. Means ± SE shown with the same letter within a column are not significantly different at $P < 0.05$

Concentration	Cytokinin	n	Total Weight (g)	Total Height (cm)	Branches per genotype
1uM	BAP	29	0.126±0.196 ^{A}	1.5±2.49 ^{D}	1.6±0.988 ^{H}
1uM	*m*T	31	0.166±0.192 ^{A}	2.0±2.29 ^{D}	2.0±0.909 ^{H}
5uM	BAP	24	0.544±0.222 ^{AB}	6.2±2.64 ^{DE}	5.4±1.048 ^{HI}
5uM	*m*T	30	1.182±0.194 ^{B}	15.2±2.31 ^{EF}	9.4±0.917 ^{J}
10uM	BAP	20	1.515±0.234 ^{BC}	25.2±2.74 ^{G}	13.0±1.088 ^{K}
10uM	*m*T	33	1.664±0.179 ^{BC}	22.4±2.13 ^{FG}	12.6±0.846 ^{K}

Table 3. The latent effect of the PGR used for multiplication on later rooting phases of micropropagation systems. Means ± SE shown with the same letter within a column are not significantly different at $P < 0.05$

Hormone	Shoots #	Rooted #	Rooting %
Run1			
*m*T	16	8	50.0
BAP	14	13	92.9
Run2			
*m*T	41	25	61.0
BAP	25	21	84.0
Total			
*m*T	57	33	57.9 ± 9.3[a]
BAP	39	34	87.2 ± 10.1[b]

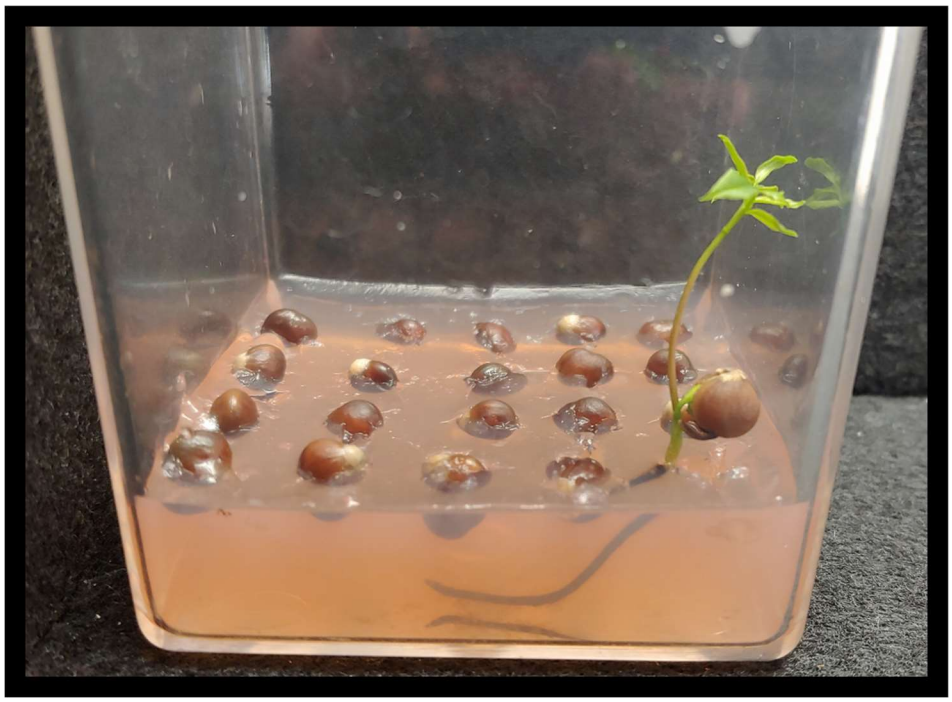

Figure 1. Long duration soaks of UCB-1 seed for germination *in vitro* A 96h soak of UCB-1 seeds in 100ppm solution of NaDCC provided adequate decontamination but was unable to provide adequate imbibition and demonstrated a low germination rate after 2 months on Pistachio Media

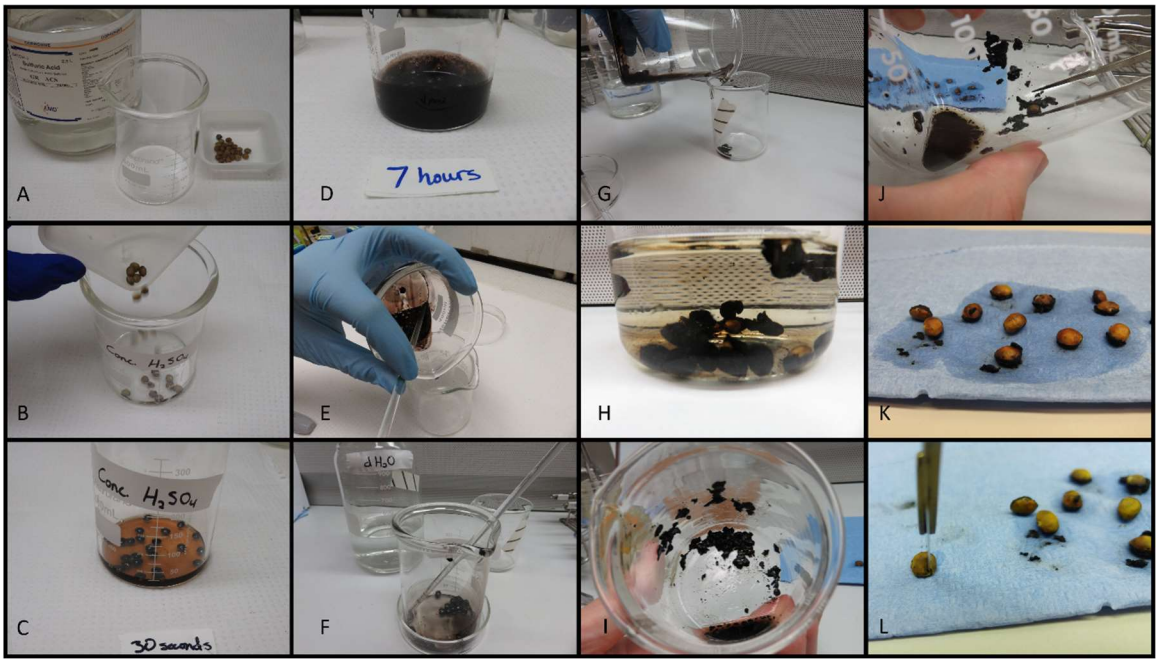

Figure 2. Acid scarification process of UCB-1 and *P. atlantica* seeds. A) 36N Sulfuric acid was poured into a beaker in a fume hood. B) clean and dry UCB-1 seed from FPS added to the sulfuric acid. C) The sulfuric acid darkened almost immediately. D) after 7 hours, the sulfuric acid was exceptionally dark and would leave a dark film when disturbed. E) decanting performed in the fume hood then transported to F) the laminar flow hood for G) transfer to sterile glassware H) and 15 minute rinses in DI water until the rinsate was clear. I) the material on the outside was removable while J) forceps were pulling seeds from the sulfuric acid. K) freshly softened and rinsed seeds placed on a sterile work surface were now able to be L) cut on the softened side for culture.

Figure 3. UCB-1 seed prepared for surface decontamination. UCB-1 seed bisected along the short axis will often retain the half of the seed containing the embryo inside the split endocarp as the seed coat is attached at the hilum end on the embryo side, while the non-embryo half is easily removed with little to no physical resistance

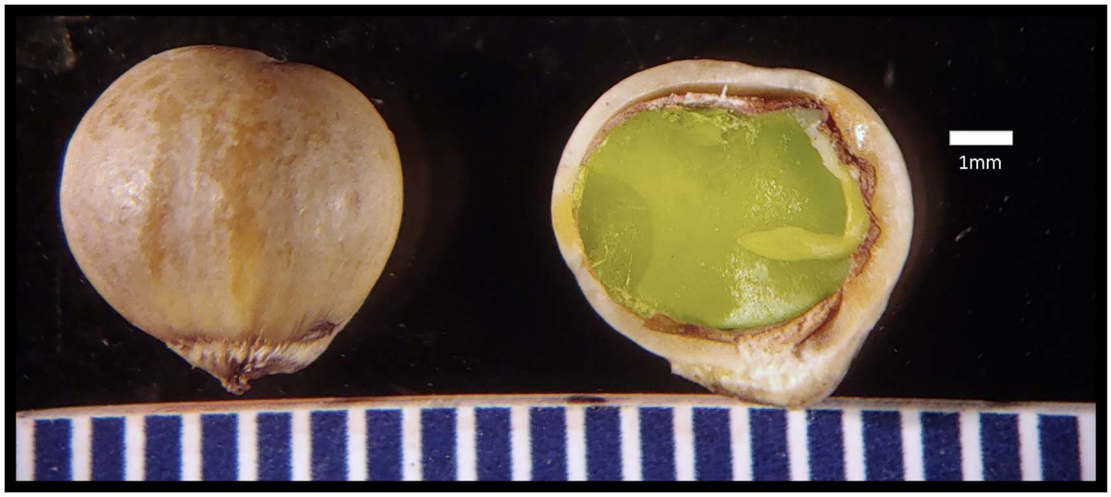

Figure 4. Intact and split endocarp displaying embryo positioning. Left, an intact endocarp with the hilum attachment skewed slightly to the embryo location on the right side of the endocarp in this view Right, a cut UCB-1 seed showing the intact embryo location with the radicle oriented toward the top of the image and away from the hilum

Figure 5. A) Intact UCB-1 seed from the front view. The non-embryo side is slightly straighter and flatter on the hilum end, with the embryo side being slightly more rounded. B) Top view of UCB-1 seed oriented in the same direction, demonstrating the slightly puckered suture on the embryo side. C) Various stages of seed removal from the endocarp all oriented with the embryo located on the right side.

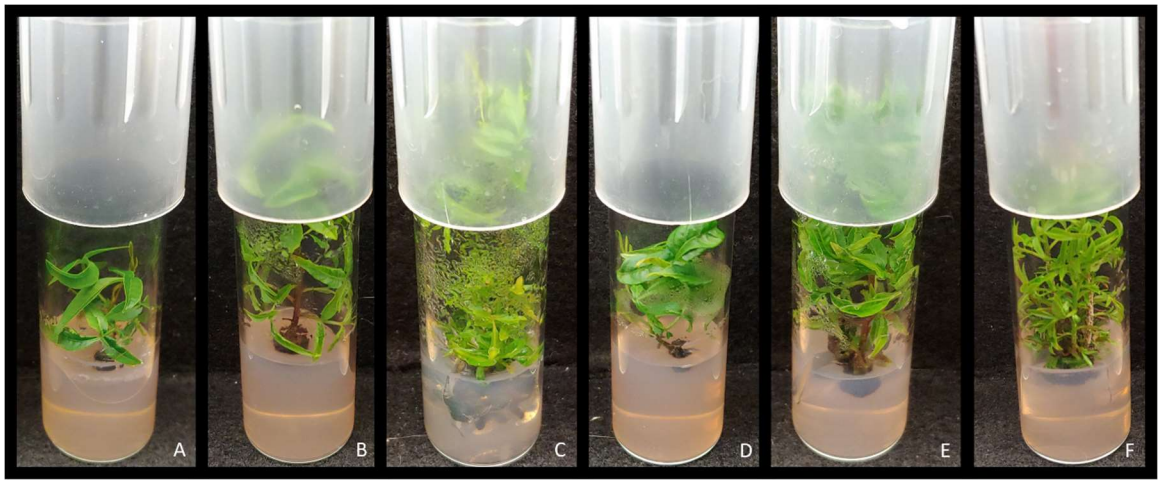

Figure 6. Representative shoot cultures from each hormone and concentration. A) 1uM BAP B) 5uM BAP C) 10uM BAP D) 1uM *m*T E) 5uM *m*T F) 10uM *m*T. For both cytokinins, the 1uM concentration provided little shoot growth. As cytokinin concentrations increase, more shoot growth and branching is observed. While some shoots, like the shoot shown in E, would have produced a taller single shoot a tube such as in F would have more shoots which would contribute to a higher total amount of shoot length available for propagation.

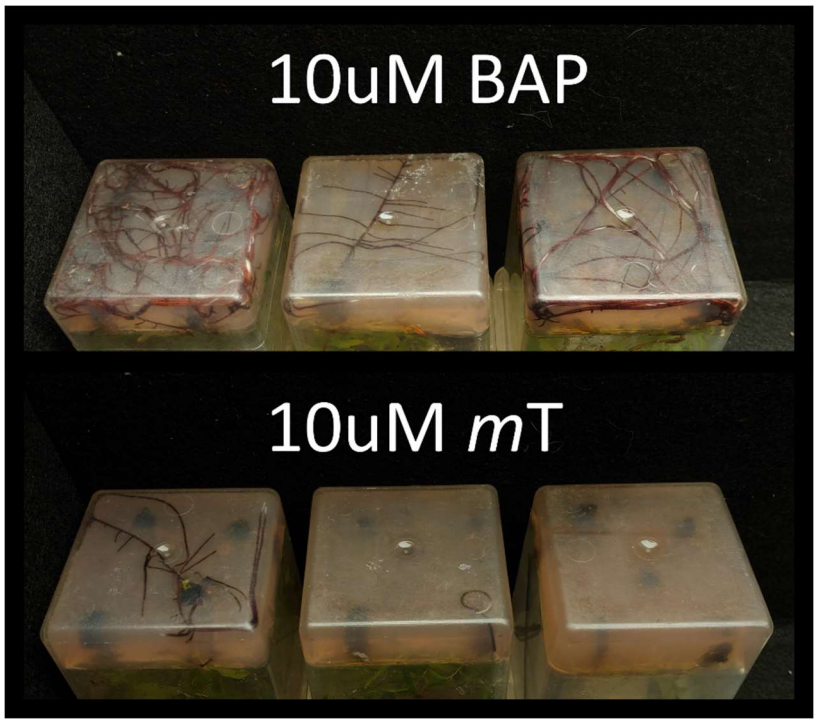

Figure 7. Latent effect of cytokinin source during multiplication on subsequent rooting phase. Both photos (Top and Bottom) were taken after 1 week induction in darkness with 50uM K-IBA followed by a 3-week expression cycle on PGR free medium. These shoots were induced and expressed on the same batches of media on the same day, and these photos were taken at the same day, 3 weeks post induction. Top, demonstrates the poorer root performance of shoots multiplied on *m*T in this study. Bottom, shows the increased percentage of rooted plants from shoots multiplied on BAP.

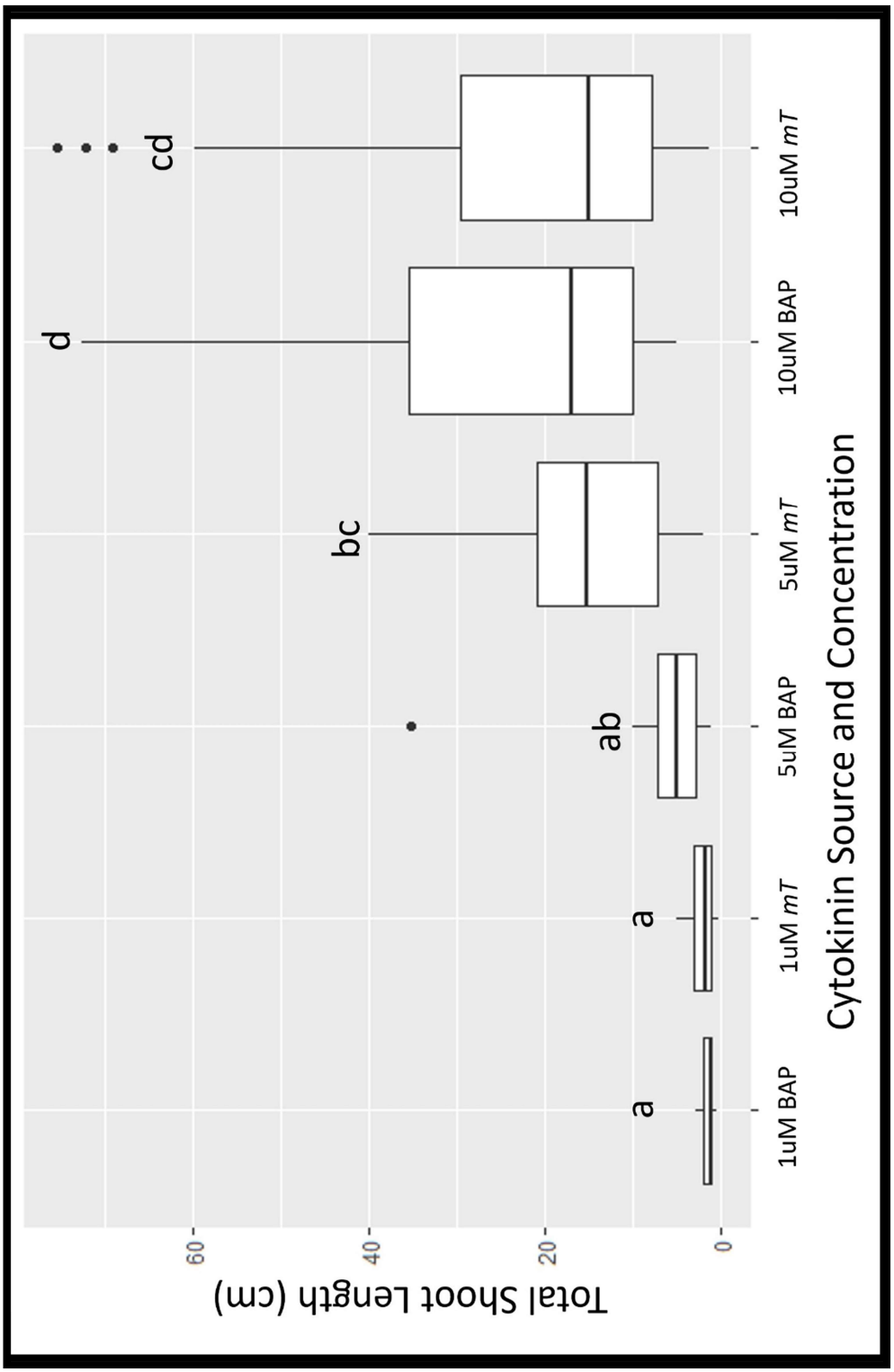

Figure 8. Total cumulative shoot length of single-shoot descent lines of UCB-1. Total cumulative shoot lengths were found to be greatest at the highest tested cytokinin concentration. More shoot length available necessarily means that more shoot sections of any given length could be cultured. Boxes in this plot shown with the same letter are not significantly different at $P < 0.05$

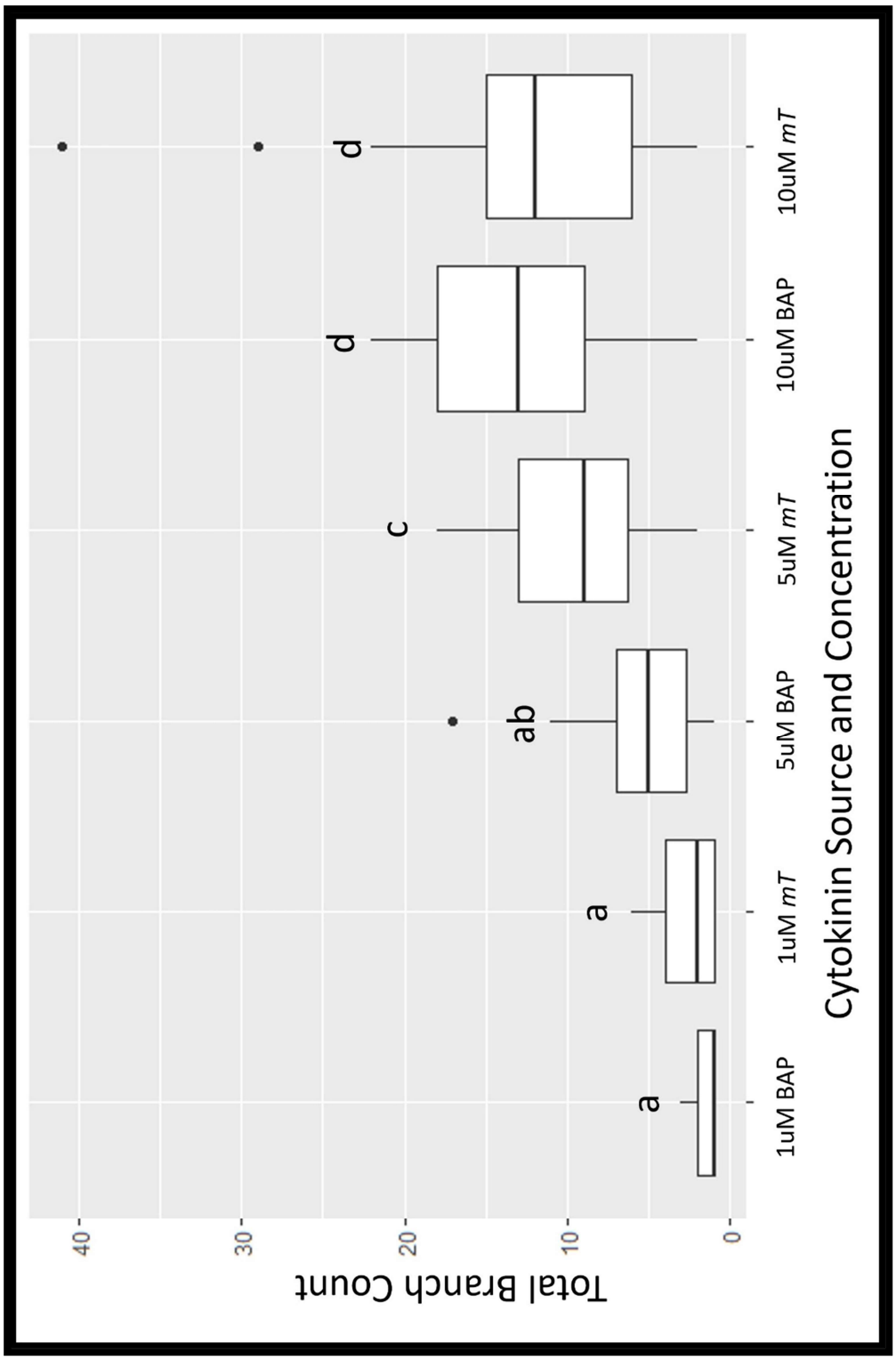

Figure 9. Effect of cytokinin source and concentration affecting the branching habit of single-shoot descent lines of UCB-1. At the 10uM concentration, both cytokinin sources were significantly more branched than shoots cultured at the 5uM or 1uM concentrations. 5uM *m*T provided significantly more branched shoot material than shoots cultured with 5uM BAP. Boxes in this plot shown with the same letter are not significantly different at $P < 0.05$

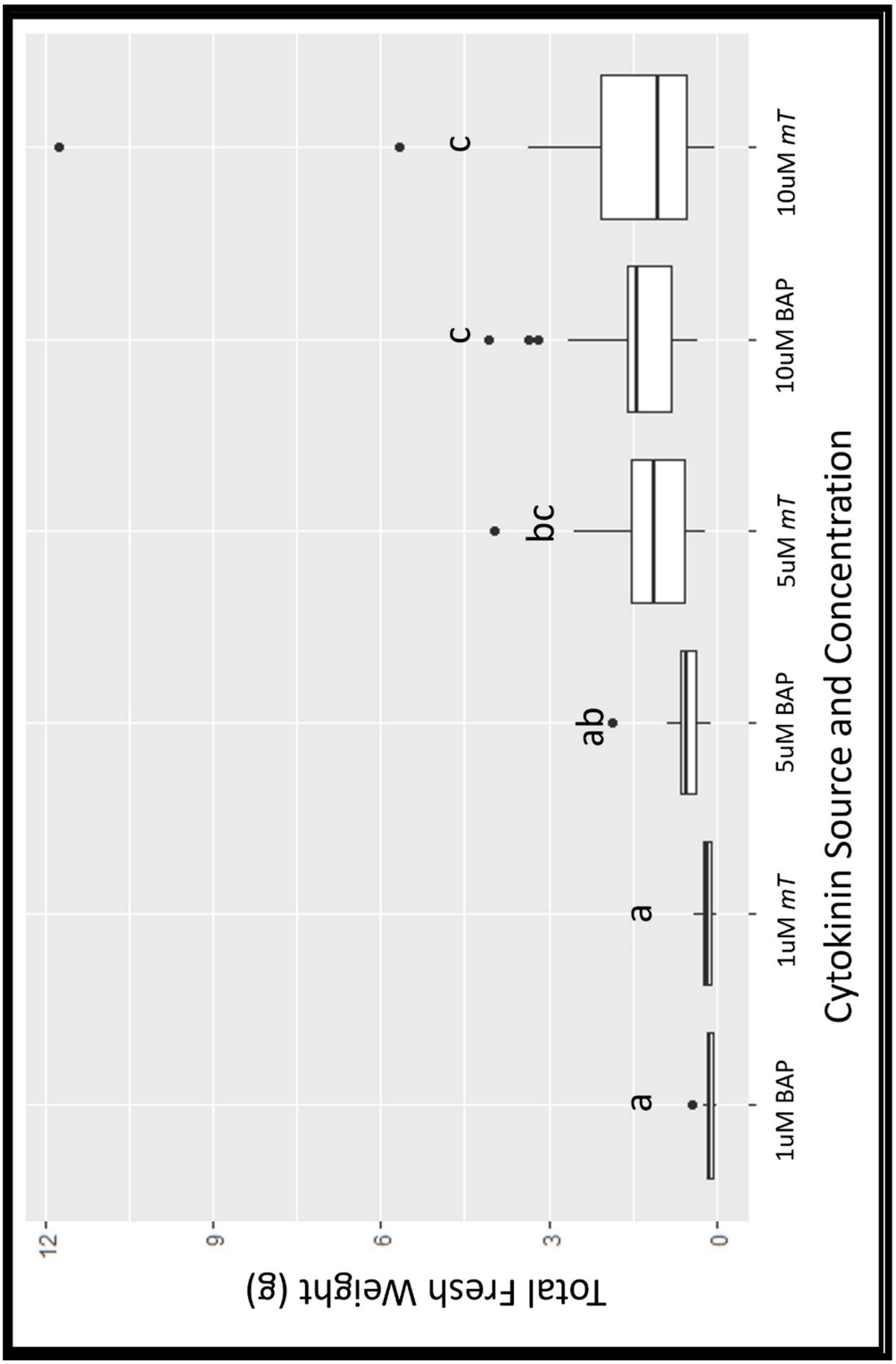

Figure 10. The combined fresh weight of each single shoot descent line. As higher concentrations of cytokinin are used, fresh weight trends higher. At 5uM, *m*T was indistinguishable from either 10uM source of cytokinin at the 0.95 confidence level. Boxes in this plot shown with the same letter are not significantly different at $P < 0.05$