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Auxin and Cytokinin Differentially Regulate Growth, Bud initiation, and Leaf Morphology in the Dryland Moss Syntrichia caninervis Brid

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Abstract

Although the role of auxin and cytokinin in growth and plant development from the cellular level to the whole plant are largely conserved across plant lineages; this does not exclude the possibility of divergence, especially in deep-splitting plant lineages. Here, the independent effects of exogenous auxin and cytokinin on the growth and development of *S. caninervis* are demonstrated, revealing concentration-dependent and antagonistic hormonal responses in development. At high concentrations, auxin promoted ectopic protonema proliferation but suppressed normal gametophore growth and pronounced disruption of leaf morphology. Cytokinin significantly enhanced bud production in a dose-dependent manner and produced comparatively modest leaf morphological changes, except for the hairpoint. In general, these findings suggest partial conservation of core auxin-cytokinin signaling mechanisms in *S. caninervis* relative to *P. patens* and vascular plants, while also revealing species-specific differences in hormonal sensitivity that likely reflect evolutionary and ecological distinctiveness of dryland mosses. From an applied perspective, the stimulatory effect of cytokinin on bud formation and/or stimulatory outgrowth protonema growth under auxin highlights the potential of targeted applications as a strategy for biocrust restoration in dryland ecosystems.

Introduction

Auxin (indole 3-acetic acid, IAA) and cytokinin, are among the most extensively studied phytohormones, recognized for their central, and often opposing roles in regulating cell division, elongation, organ initiation and morphogenesis across plant kingdom (Grobkinsky and Petrášek, 2019; Perrot-Rechenmann, 2010). Auxin acts through a nuclear signaling cascade in which auxin-dependent degradation of Aux/IAA repressors releases ARF transcription factors to activate target gene expression (Kepinski and Leyser 2005; Teh et al. 2022). Rather than acting uniformly, auxin is directionally transported via asymmetrically localized PIN efflux carriers, generating local concentration maxima that provide positional information underlying tropic responses and phyllotactic patterning at the shoot apical meristem (Reinhardt et al., 2003; Vanneste and Friml, 2009; Sassi and Vernoux, 2013). Cytokinin promotes cell division by activating CycD3 (Riou-Khamlichi et al. 1999) and maintains shoot apical meristem identity through WUSCHEL activation (Gordon et al. 2009), while it defines the boundary between meristematic proliferation and differentiation in the root (Dello Ioio et al., 2007). Acting in spatial and molecular opposition to auxin, the auxin:cytokinin ratio functions as a bistable developmental switch that determines cell fate, including the balance between meristematic proliferation and differentiation, root vs shoot organogenesis, and the positioning of organ boundaries across plant body (Enders and Strader, 2015; Schaller et al. 2015; Kieber and Schaller, 2018).

Our foundational understanding of phytohormone function in bryophytes is from studies in *Physcomitrium patens* (Rensing et al. 2020; Suzuki et al. 2021; Cammarata et al. 2023), and *Marchantia polymorpha* (Kato et al. 2020; Suzuki et al. 2023; Dipp-Álvarez et al., 2025). In bryophytes, both auxin and cytokinin regulate critical developmental transitions analogous to those observed in angiosperms (Coudert et al. 2015). Specifically in *P. patens*, auxin promotes the transition from chloronema to

caulonema, and plays a role in the initiation of shoot branching and leaf morphogenesis (Coudert et al. 2015; Thelander et al. 2018; Lin et al. 2026). Recently, it was described that PIN transporters do not create polar distribution of auxin as in vascular plants during phyllid development, rather than they reduce intracellular auxin content in all directions to reduce cell divisions and promote cell elongation across the leaf lamina (Lin et al. 2026). While cytokinin acts as a potent inducer of bud initiation from protonema, driving a rapid formation of leafy gametophore (Hata et al. 2024; Hata et al. 2025). Furthermore, auxin modulates bud formation depending on the cytokinin concentration, and the interplay between these two hormones – rather than their absolute values- is a crucial determinant of developmental outcomes in mosses (Cammarata et al. 2023). These findings highlight novel divergent mechanisms of auxin in phyllid development, but also the complexity of auxin-cytokinin crosstalk in mosses in general and reinforce the need to extend such investigations to ecologically significant species such as *Syntrichia caninervis*, for which hormonal regulation of growth remains poorly characterized.

Despite the mechanistic insights gained from *P. patens*, it remains unclear whether the hormonal responses characterized in this model species are broadly conserved across the phylogenetic and ecological diversity of mosses, particularly in species adapted to extreme environments. *S. caninervis* diverges substantially from *P. patens* in its evolutionary history, ecological context (Mishler and Oliver 2009), anatomical and morphological traits (Coe et al. 2014), and desiccation tolerance (DT) (Li et al. 2024). It has served as a model species for studying DT given its capacity to withstand near-complete cellular water loss and recover full physiological activity within minutes after rehydration (Coe et al. 2020; Silva et al. 2021). The transcriptome profiling of *S. caninervis* have dynamic changes of novel proteins during dehydration and rehydration processes, including late embryogenesis proteins (LEA), early light-inducible protein (ELIP), and production of abundant TFs during dehydration, that are crucial for maintaining cellular integrity and photoprotection, respectively (Yang et al. 2023). This finding makes this moss unique in its response to DT, raising the question of whether its response to auxin and cytokinin are similarly divergent. This uncertainty is further supported by evidence from endogenous phytohormone profiling across bryophytes, which has revealed considerable diversity of hormone metabolism, conjugation, and degradation strategies, suggesting that hormone signaling is likely to be species-specific and shaped by ecological context (Záveska Drábková et al. 2015). Extending hormonal studies to stress-tolerant species is therefore a need for more comprehensive understanding of phytohormone function across non-vascular plant lineages.

Due to its capacity to survive extreme desiccation, intense UV radiation, and drastic temperature fluctuations (Li et al. 2024), it is one of the most abundant and ecologically dominant species in forming biological soil crusts (biocrusts) of arid and semiarid ecosystems (Mishler and Oliver, 2009). With populations documented across geographically distinct regions including the Mojave Desert in United States and Gurbantunggut Desert in China, *S. caninervis*, as part of biocrust communities, are key regulators of soil stabilization, carbon cycling, and nitrogen fixation (Antoninka et al. 2016). *S. caninervis* has evolved distinctive structural and morphological traits that have direct implications in biocrust communities. Rapid leaf angle adjustments upon dehydration/rehydration processes optimizes water

uptake and photosynthetic efficiency (Wu et al. 2014). The hairpoint, a unique serrated prolongation of the midrib of the leaf, that resembling to a hair, is able to capture nanodrops (of the size of 1 nm) of water from the atmosphere (Pan et al. 2016) when fog or rain is not available. Coupled to this efficient use of water resource, perforated and elongated basal cells located at the base of the leaf also are involved in optimization of water retention and minimize water loss, further enhancing its survival in harsh desert environments (Jauregui-Lazo, et al. 2023). *Syntrichia caninervis* is particularly well-suited for studying its response to hormone application given its remarkable biology and distinctive small morphological adaptations to the world's most extreme dryland environments.

Despite the growing interest in restoring degraded dryland ecosystems and the recognized ecological importance of preserving biocrusts (Bowker et al. 2018; Eldridge et al. 2020), understanding the hormonal cues that governs *S. caninervis* growth is of both scientific and applied significance. Elucidating how auxin and cytokinin regulate gametophyte formation, protonema growth, and biomass accumulation in this species could provide valuable tools for optimizing moss cultivation for ecological restoration efforts. The cultivation of *S. caninervis* for biocrust restoration is a challenging endeavor, due to the inherently slow growth rates of dryland mosses under both natural and controlled conditions (Antoninka et al. 2016; Liu et al. 2021). While previous research has demonstrated that manipulating hydration regimes and nutrient availability (Antoninka et al. 2016), biocrust transplanting (Antoninka et al. 2022), or application of biocrust inocula in greenhouse or field conditions (Jech et al. 2025) can improve the establishment of new biocrust, the potential of phytohormone supplementation as a complementary strategy to accelerate growth rates has received little attention. Given that auxin and cytokinin are well-established regulators of cell proliferation and organ initiation in other plants (Grobkinsky and Petrášek, 2019), their targeted application could offer a novel and cost-effective approach to improving moss propagation protocols. This study therefore aims to investigate the independent effects of exogenous auxin and cytokinin treatments on the growth and development of *S. caninervis*, contributing not only to a broader understanding of phytohormone function in non-vascular plants, but also informing their potential applications in dryland ecosystem management.

Materials and Methods

Plant material and growth conditions

Shoots from *S. caninervis* collected in southern Nevada (*Stark NV-107*, USA, Nevada, Clark County,Christmas Tree Pass; UNLV) were placed into culture (Petri dish, 50 mm of diameter). To examine the hormonal regulation of growth in *S. caninervis*, cultures were established in half-strength Hoagland solution (0.5X) and incubated under controlled environmental conditions in a PHCbi humidified plant growth chamber (model MLR 352H-PA, 10.4 cu.ft.). Growth conditions consisted of a 16h light/8h dark photoperiod, day/night temperature of 23/20°C, and a photosynthetic photon flux density (PPFD) of 130 $\mu molm^{-2}s^{-1}$. These conditions were selected to promote stable protonema and gametophore growth.

Hormone treatments

To investigate the independent effects of exogenous phytohormones on gametophore growth and morphology, cytokinin and auxin were applied separately to agar plates containing Hoagland 0.5X growth medium (Hoagland's No.2 Basal Mixture, Sigma-Aldrich). The synthetic cytokinin 6-Benzylaminopurine (BAP) (Sigma-Aldrich, 3408) was supplied at 25 nM, 250 nM, and 1 µM, while the synthetic auxin naphthalene acetic acid (NAA) (Sigma-Aldrich, 1641), was applied at 100 nM, 1 µM, and 2.5 µM. A control treatment consisted of only Hoagland 0.5X medium. Our samples consisted of a single protonema mat with their subsequent developed buds and gametophores placed in a petri dish (5 cm of diameter). We analyzed a total of 30 samples (n = 30) per each treatment (Fig. 1).

Hormone concentrations were selected following Cammarata et al., 2023, who reported concentration-dependent morphological responses in *P. patens*. In contrast to the model moss, the rate of growth of *S. caninervis* is significantly slower than the model species *P. patens*. For instance, developing buds can be visible in ~ 10 to 20 days in *P. patens* under typical BCDAT growing conditions; however, *S. caninervis* develops buds in at least 45 days. Therefore, we made the decision of evaluating the morphological and anatomical traits using microscopy after 60 days. Cultures were observed at 10-day intervals for signs of contamination, and compromised plates were excluded from subsequent analysis.

Morphological and anatomical traits

To assess the growth responses of *S. caninervis* to each hormone treatment the following morphological traits were measured the after 60 days of growth. The number of buds was recorded as the count of buds that matured into a new gametophore (an individual plant bearing a stem and leaves). Protonema and gametophore growth was quantified as the percentage of the total moss cushion covered by protonema (percentage of the area covered by the final growth minus the initial growth of protonema). Leaf length (µm) was measured from the point of the leaf attachment to the stem to the tip of the lamina, excluding the hairpoint. Leaf width (µm) was measured at the widest point of the leaf lamina. Hairpoint length (µm) was measured as the hyaline cell extension that extend beyond the leaf lamina (Fig. 1). Finally, the number of cells forming the double layer (strata) in leaf cross section was recorded as count of cells forming the leaf lamina.

Statistical analysis

Overall treatment effects on protonema growth, leaf length, leaf width and hairpoint were evaluated using the Kruskal-Wallis test, and post hoc pairwise comparisons were conducted using Wilcoxon rank-sum tests (Holm method) for non-parametric data. Hormone concentration was treated as categorical factor in all analyses. Bud and cell counts were analyzed using a generalized linear model (GLM) with a Poisson distribution and hormone concentrations (BAP and NAA) as a categorical predictor. Estimated marginal means (EMMs) for each hormone treatment were calculated on the response scale, and pairwise comparisons among treatments were performed with Tukey-adjusted p-values. Predicted

means ± 95% confidence intervals were plotted alongside significance levels for each hormone treatment. Visualization of the data was performed using a boxplot with annotated significance levels in ggplot2. All statistical analysis of the data were conducted in R (v.4.5.3).

Results

Exogenous auxin and cytokinin non-linearly regulate protonema growth and bud initiation

To evaluate the independent effects of auxin and cytokinin treatment on plant growth and development, specimens were cultivated on agar media supplemented with NAA at concentrations of 100 nM, 1 µM, and 2.5 µM of NAA; and BAP concentrations of 25, 250 nM, and 1 µM (Fig. 1–2).

Auxin treatment resulted in marked protonema proliferation, as measured across all tested concentrations (Kruskal-Wallis, $p = 1e^{-05}$), with the lowest dose (100 nM) showing the greatest growth relative to the control. Surprisingly, at intermediate (1 µM) and high (2.5 µM) concentrations, protonema and rhizoids arose ectopically from leaves and stems of the gametophores (Fig. 2A). Cytokinin yielded variable effects on protonema development; however, no statistically significant BAP treatment difference was observed for protonema growth (Fig. 2B; $p = 0.34$). Both treatments did not have a significant effect on gametophore growth (Supplementary Fig. 1), except that NAA decreased slightly production of new gametophytes.

Gametophore buds arise from protonema. Auxin treatment influenced bud formation in a non-linear fashion. Most concentrations resulted in suppression of bud formation relative to the control, with the notable exception of the 1 µM concentration, where the number of buds increased approximately two-fold (Fig. 2C). In contrast, BAP treatment significantly increased bud production, with a positive relationship between cytokinin concentration and bud number. 1 µM BAP was identified as the optimum concentration for bud induction (Fig. 2D).

The filamentous growth characterized by caulonema and chloronema were found to predominate over the development of gametophores at mid to elevated auxin concentrations. The development of ectopic rhizoids was associated with the suppression of phyllid development at all auxin concentrations (Fig. 2E-G). In all BAP treatments, protonema lateral expansion, was not observed; instead, newly formed protonema was characterized by short branches of caulonema and chloronema, giving a rounded “bushy” aspect of the gametophore (Fig. 2H-J).

Auxin and cytokinin are sufficient to modulate leaf morphology

In contrast to *P. patens*, the structure of the *S. caninervis* leaf consists of a phyllid with distinct cell types, including a double stratum in cross section, perforated basal cells, and a hairpoint, most of which are crucial for survival and reproduction. To assess the influence of BAP and NAA on leaf morphology,

several quantitative morphological attributes were measured, including leaf length, leaf width, hairpoint extension, and the number of laminar cells in cross-section. At low doses, auxin treatment impacted leaf morphology, primarily reducing leaf width (Fig. 3A). At the moderate (1 µM) and highest auxin concentrations (2.5 µM), leaves were significantly shorter and narrower, accompanied by a pronounced reduction of hairpoint length (Fig. 3B-C). In contrast, cytokinin induced shorter, yet broader leaves across all tested concentrations (except 25 nM) (Fig. 3D-F), with 1 µM BAP identified as the concentration that most suppressed hairpoint development (Fig. 3F), causing significantly alteration in leaf morphology. Although, the changes in leaf morphology caused by both hormones were less pronounced at early stages of leaf development (Supplementary Fig. 2.).

Compared to the control, BAP treatment relatively produced minor morphological changes in the double strata of the lamina in cross-section that characterizes *S. caninervis* (Fig. 4A). However, a notable increase in cell number was observed at higher BAP concentrations (250 nM and 1 µM). Auxin treatment caused substantial alteration in leaf cross-section architecture (Fig. 4B). The increase in cell number caused by BAP, likely contributes to increased leaf width and stabilizing a homogenous double stratum (Fig. 4C). In contrast, auxin promoted the development of an irregular cell distribution along the lamina and a reduction in total cell number (Fig. 4D-E), leading to an irregular single stratum in cross-section and a visible wrinkled leaf phenotype (red circles in cross section, Fig. 4E).

Discussion

Divergent roles of cytokinin and auxin in protonema growth and gametophore initiation

The auxin-dependent regulation of protonema development and gametophore formation observed in *S. caninervis* is broadly consistent with developmental mechanisms characterized in *P. patens* (Suzuki et al. 2021; Cammarata et al. 2023); yet reveals concentration-dependent responses that require additional interpretation in the context of this ecologically distinct species. Similar to *P. patens*, exogenous auxin drives the transition from chloronema into caulonema cells in *S. caninervis*, accelerating colony development and expansion at low to mid concentrations (Cove and Ashton, 1984; Coudert et al. 2015). This means when exogenous auxin is present, the protonema shifts from a slow-growing, photosynthetically active state (e.g., chloronema) to a faster-growing and more exploratory filamentous form (e.g., caulonema) (Menand et al. 2007), where most of bud initiation occurs (Prigge et al. 2010; Bascom et al. 2023). In *P. patens*, this transition represents a critical developmental switch in moss growth mediated by the canonical signaling pathway, specifically via TIR1/AFB-AUX/IAA co-receptor complexes and downstream transcriptional cascades (Jang and Dolan, 2011), including PpRSL1 and PpRSL2, crucial for caulonemal identity (Prigge et al. 2010, Lavy et al., 2016). It remains to be determined if this mechanism is conserved in *S. caninervis*.

The stimulatory effect on protonema growth observed at a lower auxin concentration and the ectopic emergence of protonema and rhizoids from gametophore tissue at a high concentration (Cammarata et

al. 2023), may indicate that *S. caninervis* has reached a saturation or inhibitory threshold in the auxin signaling pathway, consistent with the species-specific hormonal responses reported in *P. patens* (Cammarata et al. 2023). In this model species, the suppression of 3D growth is understood to operate at the level of APB transcription factors, which are the molecular switch between filamentous and three-dimensional gametophore and thought to be orthologous to the *Arabidopsis* AINTEGUMENTA, PLETHORA, and BABYBLOOM (ANT/PLT/BBM) clade (Aoyama et al. 2012; Moody 2022). When AUX/IAA levels remain elevated due to sustained high auxin production in filaments, ARF transcription factors remain bound by their repressors and are unable to activate APB gene expression (Aoyama et al. 2012; de Keijzer et al. 2021). Since APB proteins are needed for cytokinin-dependent bud initiation, their absence impairs their ability to respond to cytokinin, thereby, preventing the downstream bud initiation cascade and establishment of 3D growth (Moody et al. 2022; Cammarata et al. 2023). On the other hand, the phenomenon of gametophore and phyllid outgrowth suppression and concomitant activation of filamentous and rhizoidal growth programs have been reported in *M. polymorpha* (Flores-Sandoval et al. 2015). A single knockout of the *M. polymorpha* MpTIR1 (auxin receptor) resulted in a plant unable to form a body, but which remained viable through cell proliferation, suggesting that auxin signaling is dispensable for cell survival, yet essential for organogenesis and 3D body patterning (Suzuki et al. 2023). This research establishes a parallel supporting the interpretation that the suppression of gametophore development under high NAA in *S. caninervis* reflects a disruption of auxin's role in three-dimensional developmental patterning rather than a toxic effect on cell viability.

The absence of a significant effect of cytokinin on protonema biomass ($p = 0.34$) is consistent with cytokinin primary role as a dose-dependent bud inducer rather than a protonema regulator as established in *P. patens*, and diverse moss species (von Schwartzenberg, 2007; Wang, 1981; Coudert et al. 2015) and suggests a conserved regulatority mechanism. This is mechanistically coherent with the current understanding of cytokinin signaling in mosses. Cytokinin perception activates type-B response regulators, which in turn induce APB transcription factor expression in caulonemal side-branch initials, committing them to gametophore cell fate (bud production) rather than filament elongation or chloronemal proliferation (Aoyama et al. 2012). The observation of a constant production of both short-branched caulonema and chloronema in *S. caninervis* rather than lateral expansion support this interpretation, suggesting that cytokinin redirects developmental resources toward bud initiation at the expense of continued filamentous tissue as reported in *P. patens* (Hata et al. 2024). Collectively, these data support a broader hypothesis that cytokinin-mediated bud induction represents an ancestral regulatory mechanism in land plants (Coudert et al. 2015; Hata et al. 2025). Future molecular investigations examining the expression and functional conservations of APB homologs and cytokinin signaling components in *S. caninervis* would be valuable in determining whether type B-response regulators, APB transcription factors, and downstream effectors reflect shared mechanisms or convergent evolutionary consequences to the challenge of modulating gametophore initiation. It is important to note that the morphology and structural organization of the buds produced under BAP treatment need further characterization, such as cell geometry, phyllid arrangement, and developmental

competence, may differ across BAP concentrations and influence their capacity to develop into fully functional gametophores.

Alteration of leaf morphology by exogenous auxin and promotion of laminar expansion by cytokinin

Auxin induced reductions in leaf length, width, and cell number, alongside disrupted double strata organization resulted in a 'wrinkled' leaf phenotype, suggesting that elevated exogenous auxin interferes with spatial coordination of cell division and expansion in *S. caninervis*. This potential inhibitory effect of supraoptimal auxin on leaf/phyllid morphology and cell organization are not unique to *S. caninervis*, but represent a pattern observed across phylogenetically diverse plant lineages. In *P. patens*, high NAA concentrations 2.5 μM similarly inhibited phyllid outgrowth, suppressed gametophore elongation, and produced narrow leaves (Cammarata et al. 2023). Recently, Lin et al. (2026) suggested that elevated auxin in leaves might accelerate the transition from cell division to differentiation, resulting in fewer and elongated cells across the lamina resembling to the deformed narrowed leaves of the *PINA PINB* mutant line in *P. patens*. In *M. polymorpha*, elevated exogenous auxin restricted thallus development and disrupted spatial organization of photosynthetic tissue (Suzuki et al. 2023). Among vascular plants, similar inhibitory cell expansion responses have been reported in eudicots including *Phaseolus vulgaris* (Keller, 2017) and *Arabidopsis* (Saini et al. 2017). These examples reflect a conserved inhibitory response to supraoptimal auxin concentrations across land plants and suggest that morphological disruption in *S. caninervis* is a manifestation of a deep evolutionary constraint on tolerance of high auxin concentrations in land plants. High exogenous application of NAA may also could disrupt the endogenous auxin gradients that normally coordinate the spatial pattering of cell division across leaf lamina in *Arabidopsis* (Saini et al. 2017) and *Physcomitrium* (Viaene et al. 2014; Lin et al. 2026), resulting in irregular distribution of cell in cross section and anisotropic growth that we observed in leaves of *S. caninervis*.

Cytokinin in the form of BAP increased leaf width, associated with greater laminar cell numbers at higher BAP concentrations, suggesting a proliferative effect on leaf cells consistent with cytokinin's established role in stimulating cytokinesis (Grobkinsky and Petrášek, 2019). In vascular plants, cytokinin promotes cell division by activating cyclin-dependent kinases (CDKs) and stimulates the expression of cell cycle regulators, including CYCLIN B TYPE 1 (CYCB1) and CYCLIN-DEPENDENT KINASE TYPE (CDKB) genes, through type-B transcriptional activation (Wu et al. 2021). Whether similar cell cycle regulatory mechanisms operate in *S. caninervis*, as suggested by the conservation of cytokinin signaling across land plants (Hata et al. 2025), the observed increase in laminar cell number in response to BAP treatment may reflect a cytokinin-dependent acceleration of cell division in developing leaf primordia that results in small cells and which collectively produces broader, but not necessarily longer leaves. This interpretation also needs to be validated by the orientation of cell division (anticlinal) that is associated to a particular increase of leaf width rather than coordinated cell elongation that determines leaf length. This proliferative effect of cytokinin on leaf tissue is opposite to the disruptive effects of auxin on laminar cell organization (e.g., a smaller number of laminar cells), further illustrating the antagonistic nature of auxin-cytokinin interactions in regulating leaf development in *S. caninervis*.

Cytokinin as a mode to facilitate rapid biocrust formation

The identification of 1 μM BAP as the optimal concentration for bud induction has direct and meaningful implications for the development of moss propagation protocols. Bud formation, and further moss establishment, represents a critical developmental bottleneck in moss cultivation, as buds are critical precursor for new gametophore and biomass accumulation relevant to biocrust restoration efforts (Antoninka et al. 2016). By nearly doubling bud production relative to untreated controls, this optimal concentration could substantially reduce the time required to achieve gametophores densities sufficient for effective biocrust formation and maximize developmental efficiency, potentially reducing cultivation periods as observed using biocrust inocula (Jech et al. 2025). However, the alteration of hairpoint development in response to both auxin and cytokinin treatments is of ecological relevance in a desert ecosystem. The hairpoint, a robust serrated hyaline extension of the costa, plays a critical role in the capture and harvesting of atmospheric moisture through capillary action (Pan et al. 2016). Therefore, the hormonal imbalance caused by application of exogenous hormones could compromise key adaptive traits that influence survival of *S. caninervis* under cultivation conditions. Therefore, several practical considerations require attention before translating these findings to large-scale restoration. First, the long-term effect of BAP application on gametophore morphology, physiology, and desiccation tolerance, traits that are essential for survival in desert ecosystem, like the hairpoint, remain unknown and require evaluation in future studies. Second, the interaction between endogenous cytokinin and exogenous BAP supplementation is poorly characterized and the potential hormonal imbalance to produce alteration in development at the whole moss cushion need careful consideration under controlled and field conditions. Finally, the combined application of BAP concentrations with complementary growth-enhancing strategies, such as moderate auxin supplementation shown here to stimulate protonema proliferation, represents a promising avenue of research for developing integrated hormonal protocols that simultaneously accelerate both filamentous and gametophore stages in *S. caninervis* which ultimately contribute to restoration programs. Further research should systematically vary the ratio of auxin and cytokinin, employ mutant lines, and overexpression of genes, should be attempted to understand hormonal regulation in *S. caninervis* and identifying optimal hormonal conditions for maximizing biomass, while stabilizing morphological attributes, and the preservation of ecologically critical biocrust.

Declarations

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Author Contribution

Conceptualization: JJL and SMB; Methodology: JJL, IL, and SMB; Analysis: JJL and IL; Data Curation: JJL; Writing original draft: JJL and SMB; Funding acquisition: JJL and SMB.

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Availability of data and materials

The datasets generated and analyzed during the current study are available in Dryad. DOI: 10.5061/dryad.k0p2ngfqj

Competing interests

The authors declare that they have no competing interests.

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Artificial Intelligence

During the preparation of this work, the author(s) used Claude AI (Anthropic, accessed June 2026) to create a list of top references related to the use of auxin and cytokinin in plants. After using this tool, the authors reviewed and verified the content as needed and take full responsibility for the accuracy and integrity of the publication.

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Figures

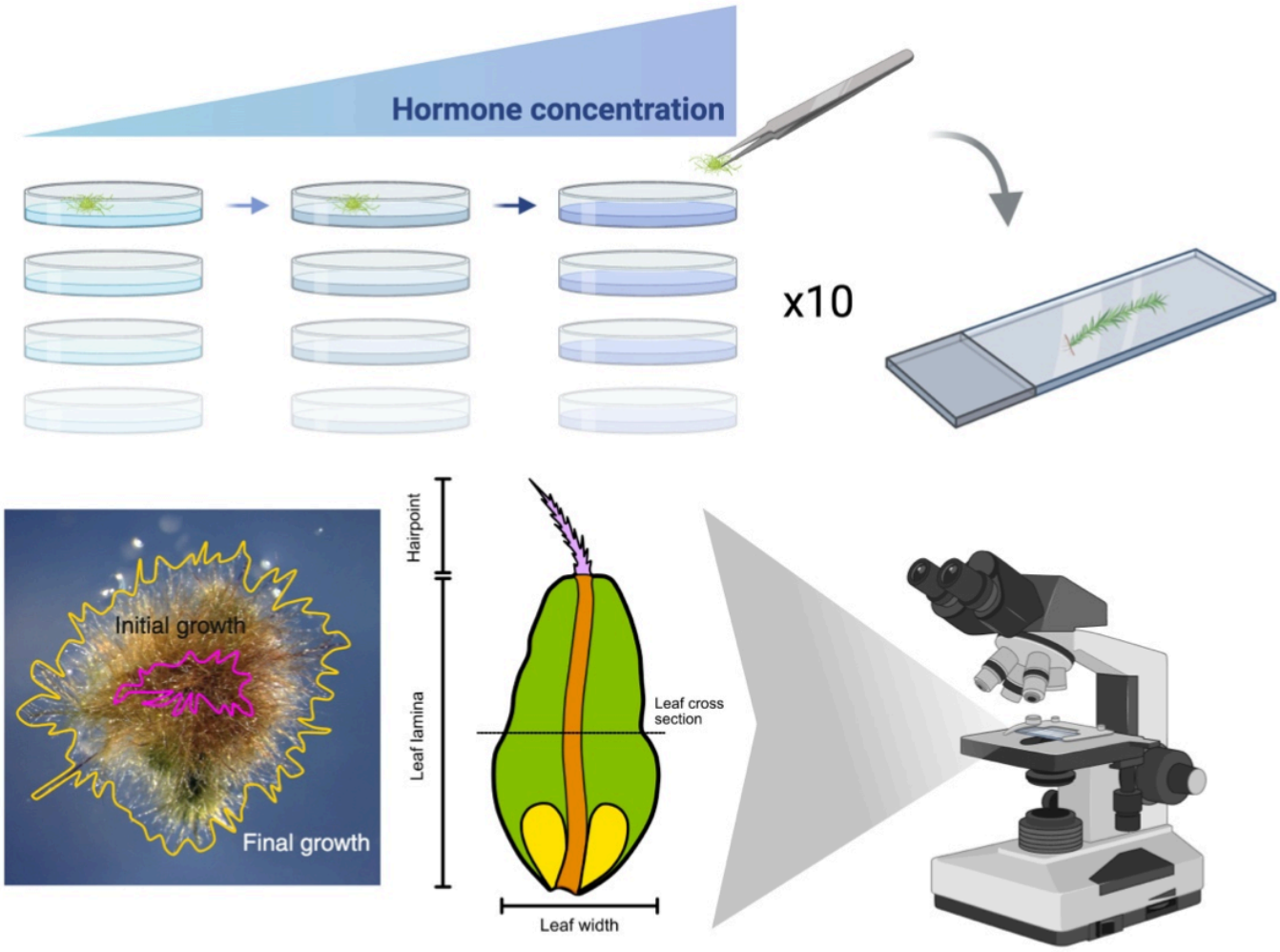

Figure 1

Experimental setup for the individual application of cytokinin 6-Benzylaminopurine (BAP) and naphthalene acetic acid (NAA) treatments. Plants were placed on growth medium under controlled conditions. Gametophore features (bud count and percentage of protonema growth) and leaf

morphological traits (leaf length, width, hairpoint, and cell distribution in cross section) were measured after 60 days of cultivation. This figure was designed using Biorender.com

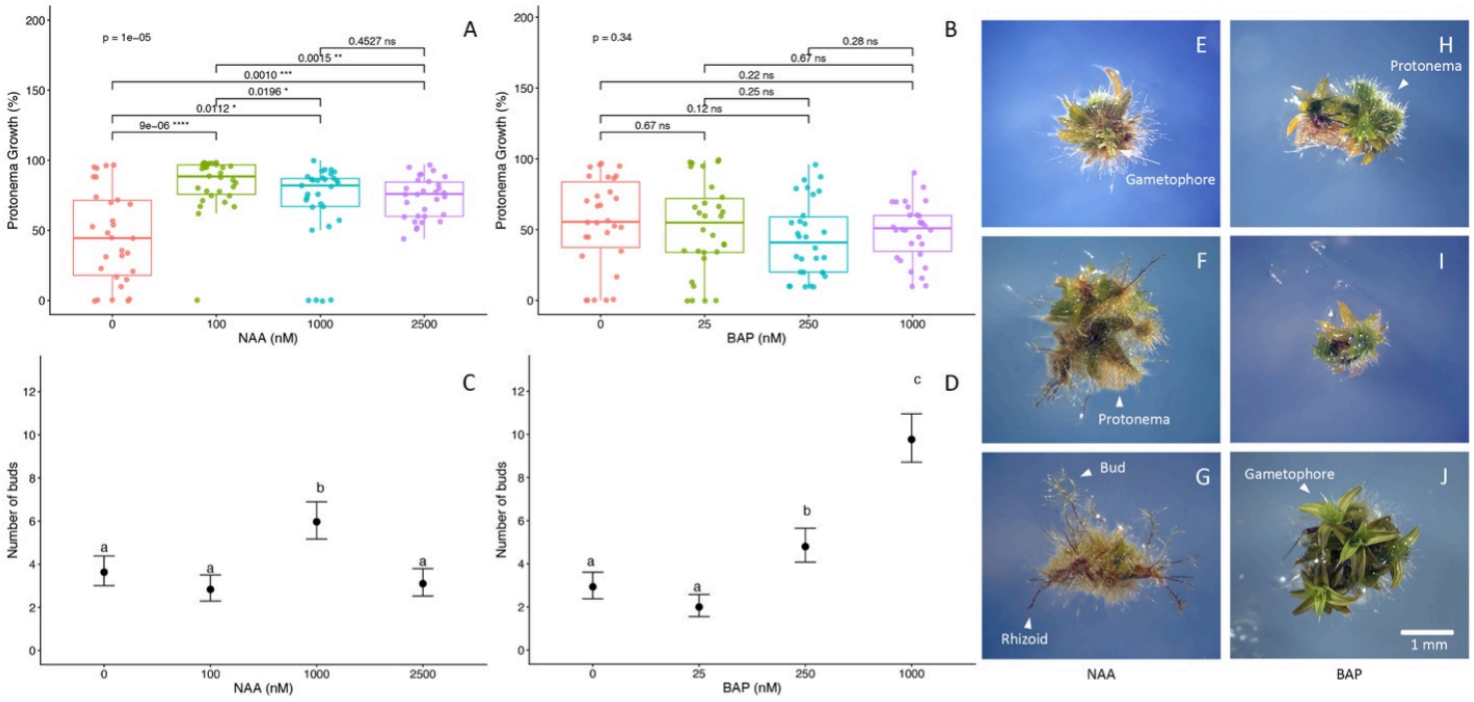

Figure 2

Syntrichia caninervis gametophore and protonema respond in a dose-dependent fashion to exogenous NAA and cytokinin. Protonema growth (%) (A-B) according to different NAA and BAP concentrations. Pairwise differences between concentrations were assessed using Wilcoxon tests (annotated brackets), and overall differences were evaluated with Kruskal-Wallis (top left p-value). Number of buds (C-D) derived from a Poisson generalized linear model (GLM) are shown for each growth in each NAA and BAP concentration. Values represent model-based expected means (+- 95 CI) with Tukey-adjusted comparisons among the levels. Plants were treated with NAA (E: 100 nM; F: 1 mM NAA; G: 2.5 mM) or BAP (H: 25 nM BAP; I: 250 nM BAP; and J: 1 mM BAP) independently and imaged after 60 days.

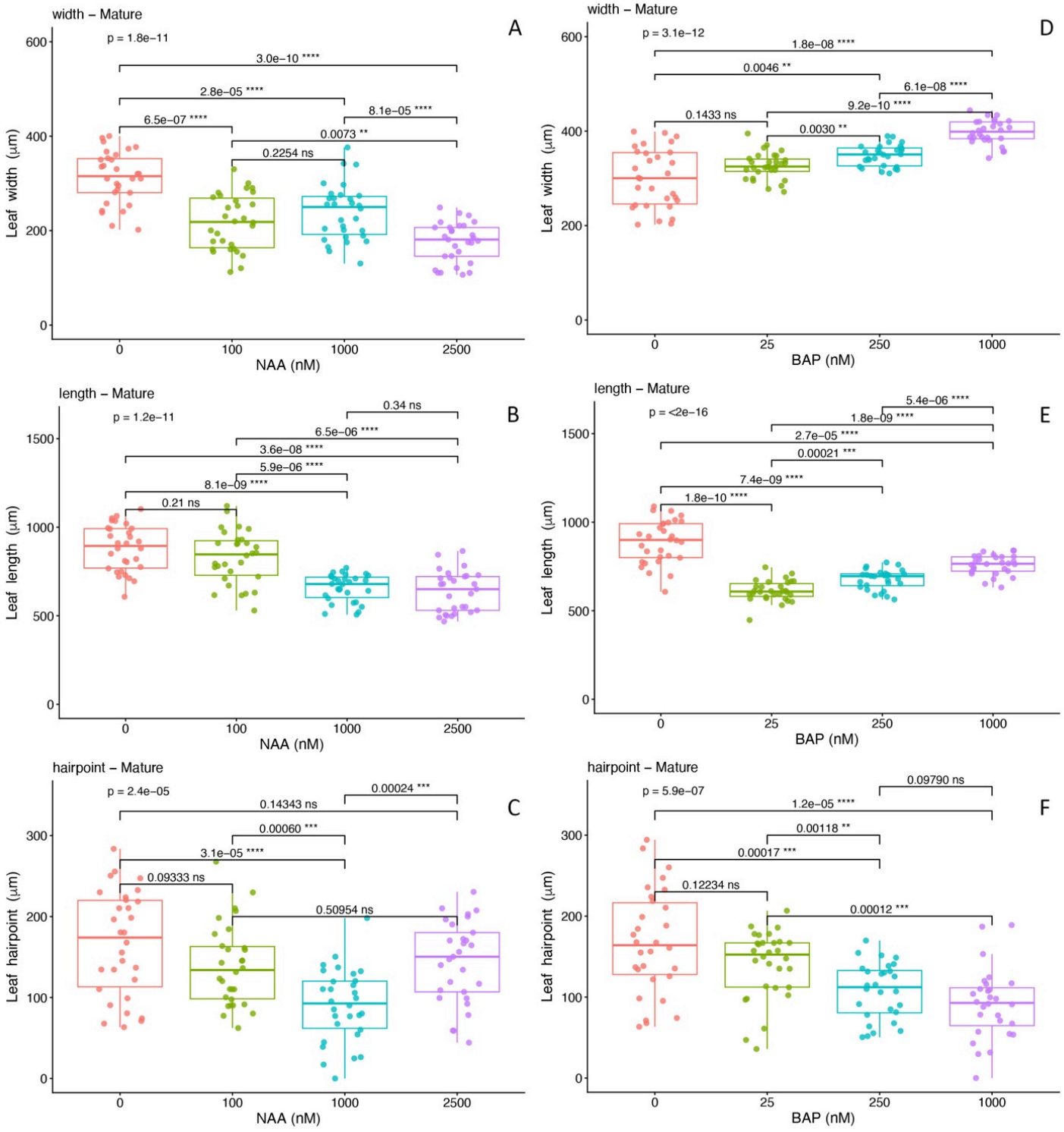

Figure 3

Alterations in leaf morphology caused by different concentration of NAA and BAP. Boxplots showing the effect of exogenous NAA (A-C) and BAP (D-F) on leafy gametophore parameters, including leaf width, leaf length, and extension of the hairpoint measured after 60 days of treatment. Overall differences were evaluated with Kruskal-Wallis (top left p-value), while posthoc test differences among concentrations were assessed using Wilcoxon test (annotated brackets).

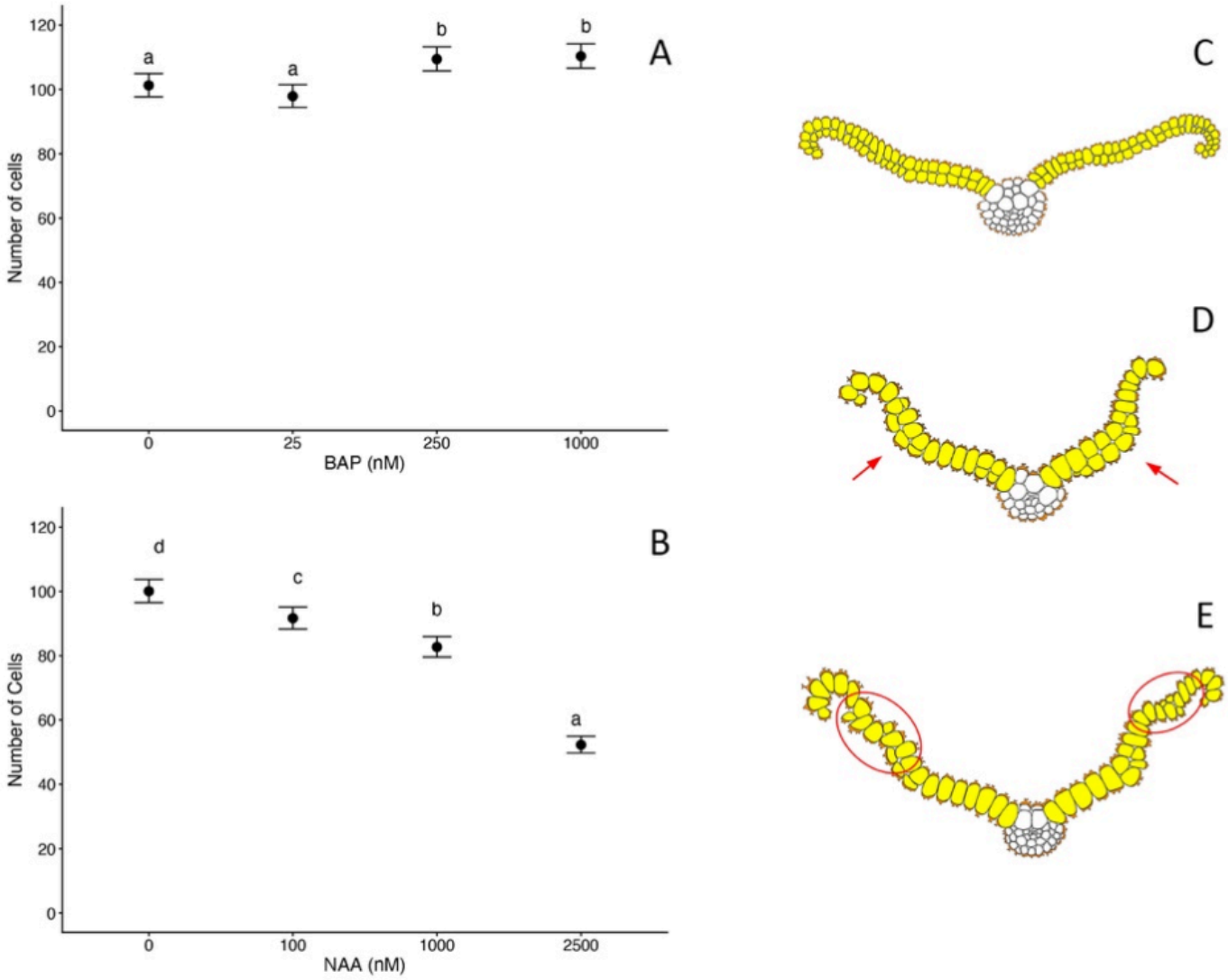

Figure 4

Opposing effects of NAA and BAP on cell count and ornamentation of leaf cross-section. Estimated number of cells shown in cross-section according to the different levels of BAP (A) and NAA (B) concentrations. Levels of significance (letters) were estimated from a Poisson generalized linear model (GLM) for each NAA and BAP treatment. Values of number of cells represent model-based expected means (+- 95 CI) with Tukey-adjusted comparisons among the treatments. Diagrams of a typical (C), irregular (D), and abnormal (E) distribution of the cells along the double strata in cross-section (arrows indicate the irregular formation of the double strata, while ovals show the ornamentation of the cells that results in a wrinkled phenotype. Only laminar cells shown in yellow were counted.

Supplementary Files

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