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Plant growth regulators for enhancing Alberta native grass and forb seed germination

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ABSTRACT

A germination trial was conducted to screen effects of plant growth regulators (PGRs) on 9 Alberta native grass and forb species with the aim of identifying PGRs with the capacity to improve seed germination and early plant development in disturbed and reconstructed soil conditions. Seeds were treated with 500 mg/L of various gibberellins (GA_3_40 [40% GA_3], GA_3_90 [90% GA_3], and $GA_{4/7}$), 5 mg/L cytokinin (kinetin), and 0.1 mg/L brassinosteroids (brassinolide). Experiments were conducted in a growth chamber following a 24 h soaking period. PGR seed treatment did not significantly increase percent (%) germination for the majority of species, but rather assisted in breaking seed dormancy and enhancing early radical emergence. Early germination at day 7 was measured for *Fragaria virginiana* (130–150% increase over the control for GA_3_40, GA_3_90, $GA_{4/7}$, and brassinolide), *Koeleria macrantha* (36% increase for GA_3 40), *Poa palustris* (98–123% increase for all PGRs), *Agrostis scabra* (42–56% increase for all PGRs) and *Festuca hallii* (85–93% increase for kinetin and brassinolide). Gibberellin treatments were significantly more effective in improving shoot growth; kinetin and brassinolide were significantly more effective in enhancing root development for the majority of tested plant species. PGRs having the greatest overall impact on seed germination and plant development, as measured by vigor index were brassinolide and $GA_{4/7}$. Tested PGRs have the potential to benefit native grass and forb restoration and revegetation efforts, improving the efficacy of planting prescriptions, with the aim of increasing early ground cover, stabilizing soils, enhancing biodiversity, and reducing the time to reclamation certification.

1. Introduction

Alberta, Canada is known for its diverse terrain and ecoregions, ranging from mountain- to prairie grass-dominated landscapes (Downing and Pettapiece, 2006). The increase in industrial activity across the province (e.g., oil and gas extraction and transportation, mining, etc.) since the 1990s has led to an increase in reclamation and revegetation projects, each with their own unique climatic, soil, physiographic, and anthropogenic-influenced site-specific challenges. There has been a push towards the development of innovative, practical, sustainable, and cost-effective revegetation practices that help ensure reclamation after resource development activities. The goal is to produce self-sustaining communities in alignment with regulated end goals. One of the major challenges includes the enhancement of native seed establishment through the breaking of seed dormancy, improving germination percentage, and increasing below- and above-ground biomass development, in reclaimed, reconstructed and/or modified soils (Schoonmaker et al., 2014). The production of high quality seedlings from native seed in commercial tree nurseries has also been found to be challenging, owing to non-uniform seed germination and poor root development within plugs (MacDonald et al., 2015).

This paper explores the exogenous application of plant growth regulators (PGRs) under controlled laboratory conditions, as a method for improving the germination and early establishment of native species. Some PGRs are chemical stimulants (or synthetic analogues) that mimic natural plant hormones, while others (i.e. GA_3, GA_4 and GA_7) are naturally occurring plant hormones synthesized through the fermentation process. They all work by promoting existing plant hormonal activity by stimulating or inhibiting enzymes or enzyme systems. PGRs have been commonly used throughout agriculture, viticulture and horticulture to improve seed germination, plant growth and yield under non-ideal, stressful conditions (e.g., low soil fertility, disease, short growth seasons) (Harms and Oplinger, 1988; Mitchell, 1942; Rademacher, 2015; Small and Degenhardt, 2018; Weintraub and Norman, 1949) – similar to conditions experienced in land reclamation scenarios. By modifying hormonal activity, PGRs have been measured

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Table 1
Alberta native grass and forb species selected for the trial and their associated predominant Natural Region (Downing and Pettapiece 2006).

Plant Type	Common Name	Scientific Name	Predominant Natural Region
Grass	Western Wheatgrass	*Pascopyrum smithii*	Grassland
	Hairy Wild Rye	*Elymus innovatus*	Boreal Forest, Foothills, Rocky Mountain
	June Grass	*Koeleria macrantha*	Boreal Forest, Foothills, Rocky Mountain
	Fowl Bluegrass	*Poa palustris*	Boreal Forest, Foothills
	Ticklegrass	*Agrostis scabra*	Boreal Forest, Foothills
	Plains Rough Fescue	*Festuca hallii*	Grassland
	Rocky Mountain Fescue	*Festuca saximontana*	Boreal Forest
Forb	Wild strawberry	*Fragaria virginiana*	Boreal Forest
	Yarrow	*Achillea millefolium*	Foothills, Grassland, Parkland

to beneficially improve both seed germination and seedling growth (Harms and Oplinger, 1988; Hopkins and Hüner, 2004). Therefore, it is proposed that PGR application to native grass and forb seeds may enhance seed germination and establishment.

2. Materials and methods

A germination experiment was conducted at the InnoTech Alberta Greenhouse Complex (Vegreville, AB) on seven native grass species and two native forb species (Table 1), using five commercially available PGR products. Plants were selected to capture a range of predominant grass and forb species commonly found in five out of six Natural Regions in Alberta including: Grassland, Foothills, Rocky Mountains and Boreal Forest. Seeds were obtained from the wild seed collection at the InnoTech Alberta Native Seed Bank.

Products tested included the following commercially available PGRs: gibberellin (GA_3; ProGibb 40SG® Valent BioSciences LLC and $GA_{4/7}$; ProVide®10SG Valent BioSciences LLC), kinetin (cytokinin; Gold Bio Kinetin), and brassinolide (brassinosteroid; Power Grown Brassinolide 0.2%) (George et al., 2008; Greippsson, 2001; Gupta and Chakrabarty, 2013; Rao et al., 2002). For GA_3, two different concentrations of the active ingredient were tested, containing either 40% (GA_3_40; ProGibb 40SG ® Valent BioSciences LLC) or 90% (GA_3_90; MP Biomedicals Gibberellic Acid) GA_3.

For all gibberellin treatments, seeds were soaked in 20 mL of 500 mg/L GA_3_40, GA_3_90, or $GA_{4/7}$ for 24 h at 20 °C; gently swirled at the beginning, middle and end of the soaking period (Machado de Mello et al., 2009). Seeds were then filtered and rinsed under ultra-pure water for 2 min. Similar methodology was used for kinetin and brassinolide treatments using 5 mg/L and 0.1 mg/L solutions, respectively, without rinsing following the 24 h soaking period (Anuradha et al., 2002; Sawan et al., 2000). For comparison, a hydropriming treatment including soaking seeds in 20 mL ultra-pure water for 24 h, and a control treatment including the germination of dry seeds (direct from cold, dry storage) were included for each plant species.

For each plant species, twenty seeds from each treatment were placed in petri plates containing two sheets of Whatman No. 1 filter paper and saturated with ultra-pure water. Each treatment included five replicates (100 seeds in total). Plates were incubated in a germination cabinet at 21 °C day/18 °C night for 10/14 h light/dark for a period of 14 days. Humidity was maintained at 80% throughout the duration of the experiment. Ultra-pure water was added to petri plates, as required, every 2 to 3 days to maintain adequate moisture.

Germination percentage was recorded on day 7, indicated by the emergence of the radical through the seed coat. On day 14, germination percentage, shoot length and root length were recorded. Vigor index was calculated as per Chithrashree et al. (2011): percent germination × (mean root length + mean shoot length).

Data were tested for normality using the Shapiro-Wilk Normality Test. Statistical significance was assessed using a generalized linear mixed model with treatments as fixed effects and replicates as random effects comparing germination, vigor, shoot length, and root length. Differences were considered to be significant when the probability value was less than 5% ($p < 0.05$). Tukey's Honestly Significant Difference (HSD) test, a multiple-comparison procedure, was used to assess whether treatment means were statistically significantly different from one another. Data were analyzed in JMP® (SAS institute).

3. Results

3.1. Seed germination percentage

PGRs significantly increased the mean germination percentage of *F. virginiana*, *P. palustris*, *A. scabra* and *F. hallii* compared to their control treatments; no observable patterns were found between species (Table 2). The germination between the control and the hydropriming treatment were not significantly different, moreover a number of species (*F. virginiana* and *A. scraba*) were observed to have a lower 14 day germination compared to the control. For *F. virginiana*, the following PGRs increased the mean germination percentage of seeds at day 7 compared to the control and hydropriming treatments: GA_3_40 (130%), GA_3_90 (131%), $GA_{4/7}$ (132%) and brassinolide (149%). PGR treated seeds exhibited higher germination at day 7 compared to the control treatment, *P. palustris* (all PGRs), *A. scabra* (GA_3_40, GA_3_90, $GA_{4/7}$) and *F. hallii* (kinetin and brassinolide only). On day 14, the total number of germinated *F. hallii* exceeded the control by 77% for seeds treated with kinetin on day 14.

3.2. Shoot and root length

At least one of the PGR seed treatments stimulated shoot growth for all forbs and grasses by day 14, with the exception of *E. innovatus* (Fig. 1). PGRs associated with the majority of significant increases in shoot growth for the grasses included $GA_{4/7}$ (five species), GA_3_40 (four species), and GA_3_90 (three species). The increase in the concentration of GA_3 from 40% to 90% did not positively increase shoot growth with the exception of *F. hallii*. For *F. hallii*, while the GA_3_90 treatment was not significantly different from control, the GA_3_40 treatment had a positive significant effect on shoot length. For the forb species, the most significant ($p = 0.05$) improvements in shoot growth were measured for *A. millefolium* treated with GA_3_40 and $GA_{4/7}$ (5.8 mm and 5.1 mm) – two times that of the control (2.5 mm). Shoot growth in *F. virginiana* also significantly increased with $GA_{4/7}$ (5.1 mm) in comparison to the control (1.7 mm).

PGRs associated with the majority of significant increases in root growth included brassinolide (six species), kinetin (five species), GA_3_40 (four species), and GA_3_90 (two species). The greatest measureable increase in root length was two times the control for *A. millefolium* (23.3 mm and 27.6 mm/11.6 mm control) and *F. virginiana* (12.1 mm and 13.2 mm/4.7 mm control), and three times that of the control for *A. scabra* (16.6 mm and 18.4 mm/4.7 mm control) for both kinetin and brassinolide after 14 days.

Table 2
Effect of plant growth regulator (PGR) treatments on seed germination (day 7 and 14). Numbers in bold indicate a significant difference over the control treatment for that species.

Plant Species	PGR Treatment	Mean Germination %)	
		Day 7	Day 14*
A. millefolium	Control	93^{a}	94^{a}
	Hydroprime	87^{a}	93^{a}
	GA_3_40	89^{a}	89^{a}
	GA_3_90	96^{a}	96^{a}
	$GA_{4/7}$	91^{a}	91^{a}
	Kinetin	82^{a}	82^{a}
	Brassinolide	95^{a}	96^{a}
F. virginiana	Control	0^{a}	72^{a}
	Hydroprime	0^{a}	**6^{b}**
	GA_3_40	**30^{bc}**	75^{a}
	GA_3_90	**31^{bc}**	66^{a}
	$GA_{4/7}$	**32^{bc}**	76^{a}
	Kinetin	13^{ab}	87^{a}
	Brassinolide	**49^{c}**	83^{a}
P. smithii	Control	86^{a}	89^{a}
	Hydroprime	**66^{b}**	**69^{b}**
	GA_3_40	81^{ab}	85^{ab}
	GA_3_90	79^{ab}	83^{ab}
	$GA_{4/7}$	83^{a}	84^{ab}
	Kinetin	82^{ab}	83^{ab}
	Brassinolide	79^{ab}	86^{a}
E. innovatus	Control	40^{abd}	59^{a}
	Hydroprime	**16^{c}**	46^{a}
	GA_3_40	29^{abd}	38^{a}
	GA_3_90	27^{a}	46^{a}
	$GA_{4/7}$	31^{abd}	39^{a}
	Kinetin	44^{d}	64^{a}
	Brassinolide	35^{abd}	58^{a}
K. macrantha	Control	55^{ab}	79^{a}
	Hydroprime	40^{b}	57^{a}
	GA_3_40	75^{a}	76^{a}
	GA_3_90	62^{ab}	71^{a}
	$GA_{4/7}$	70^{a}	72^{a}
	Kinetin	65^{ab}	72^{a}
	Brassinolide	68^{ab}	78^{a}
P. palustris	Control	39^{a}	81^{a}
	Hydroprime	**87^{b}**	87^{a}
	GA_3_40	**87^{b}**	89^{a}
	GA_3_90	**77^{b}**	81^{a}
	$GA_{4/7}$	**84^{b}**	87^{a}
	Kinetin	**85^{b}**	86^{a}
	Brassinolide	**86^{b}**	89^{a}
A. scabra	Control	57^{ab}	73^{a}
	Hydroprime	43^{a}	44^{b}
	GA_3_40	82^{bc}	84^{a}
	GA_3_90	**86^{c}**	88^{a}
	$GA_{4/7}$	81^{bc}	82^{a}
	Kinetin	**89^{c}**	92^{a}
	Brassinolide	85^{bc}	85^{a}
F. hallii	Control	27^{a}	55^{a}
	Hydroprime	**45^{b}**	71^{ab}
	GA_3_40	33^{a}	47^{a}
	GA_3_90	28^{a}	68^{ab}
	$GA_{4/7}$	40^{ab}	67^{ab}
	Kinetin	**50^{b}**	**77^{b}**
	Brassinolide	**52^{b}**	66^{ab}
F. saximontana	Control	84^{ab}	89^{a}
	Hydroprime	97^{a}	96^{a}
	GA_3_40	87^{ab}	93^{a}
	GA_3_90	92^{ab}	94^{a}
	$GA_{4/7}$	82^{ab}	99^{a}
	Kinetin	78^{b}	82^{a}
	Brassinolide	96^{a}	97^{a}

* Means within plant species followed by the same letter are not significantly different at $p \geq 0.05$ for each parameter between treatments. Numbers in bold indicate a significant difference over the control treatment for that species.

3.3. Vigor index

Vigor index results between treatments were significantly different for all species except for *E. innovatus* and *F. hallii*. The species that responded most consistently to PGR treatments compared to control and/or hydropriming were: *A. millefolium* and *A. scraba* (Fig. 2). For *F. virginiana*, vigor significantly increased for seeds treated with kinetin and brassinolide in comparison to the control and hydropriming; the differences between kinetin and brassinolide treatments were not significant.

For the grasses, the treatment(s) associated with significant increases in vigor index over the control and/or hydropriming for *P. smithii* was GA_3_90 and $GA_{4/7}$; GA_3_40 and $GA_{4/7}$ for *K. macrantha*; GA_3_90 and $GA_{4/7}$ for *P. palustris*; all PGRs for *A. scabra*; and brassinolide for *F. saximontana*.

4. Discussion

For the majority of species, PGR seed treatment did not significantly increase the total number of seeds germinated by day 14, but rather assisted in breaking seed dormancy and early radical emergence at day 7. This application of PGRs may be beneficial for extending the longevity of seed stocks, known to decrease in viability over time regardless of the storage conditions.

Overall, the significant increases in shoot growth for the grasses and forb species tested in this study were associated with gibberellin treatment (GA_3_40, $GA_{4/7}$, and GA_3_90). The gibberellins had a larger overall positive impact on early shoot development across all the native plant species. Gibberellins are well known for their capacity to control cell elongation and stem internode elongation; cell division in plant shoots is directly influenced through the stimulation of ribonucleic acid and protein synthesis (George et al., 2008; Gupta and Chakrabarty, 2013; Hamayun et al., 2010; Harms and Oplinger, 1988; Kaur et al., 1998). Hydropriming increased shoot length in *F. hallii* compared to the control; the increase was similar to the GA_3_40 and GA_3_90 treatments. Kinetin and brassinolide were not observed to have any impacts on shoot growth but were found to have a greater influence over early root development. This was expected, owing to the modes of action of the specific PGRs. Cytokinins, including kinetin, are naturally produced in developing or meristematic tissues and organs, including root tips (Osugi and Sakakibara, 2015; Rademacher, 2015). Cytokinins counteract auxins in apical dominance (or central stem growth) and promote lateral plant growth, resulting in the production of lateral shoots and roots (Rademacher, 2015). They have also been found to enhance root growth rate (Carrow and Duncan, 2012). Brassinosteroids, including brassinolide, have been measured at highest concentrations within plant reproductive organs and growing tissues, including roots (Khripach et al., 2000). Their presence has been found to influence and affect growth and developmental processes including cell elongation (Khripach et al., 2000).

In general, brassinolide had a greater overall influence in root length; $GA_{4/7}$ and GA_3_90 had a greater overall influence on shoot length. Given the different modes of action of the various PGRs and its corresponding impact on either germination, shoot or root development, it is challenging to rank the efficacy of the product early seedling emergence. Hence, a combined performance measure such as the vigor index, which includes the three performance variables that were monitored (germination, shoot and root length), is an inclusive indicator for PGR efficacy. Based on the calculated vigor index, gibberellin treatments (GA_3_90 and $GA_{4/7}$) and brassinolide had the greatest impact on early seedling emergence across the species tested.

5. Conclusions

The exogenous application of PGRs as seed soaks had a positive impact on seed germination and early plant growth and development

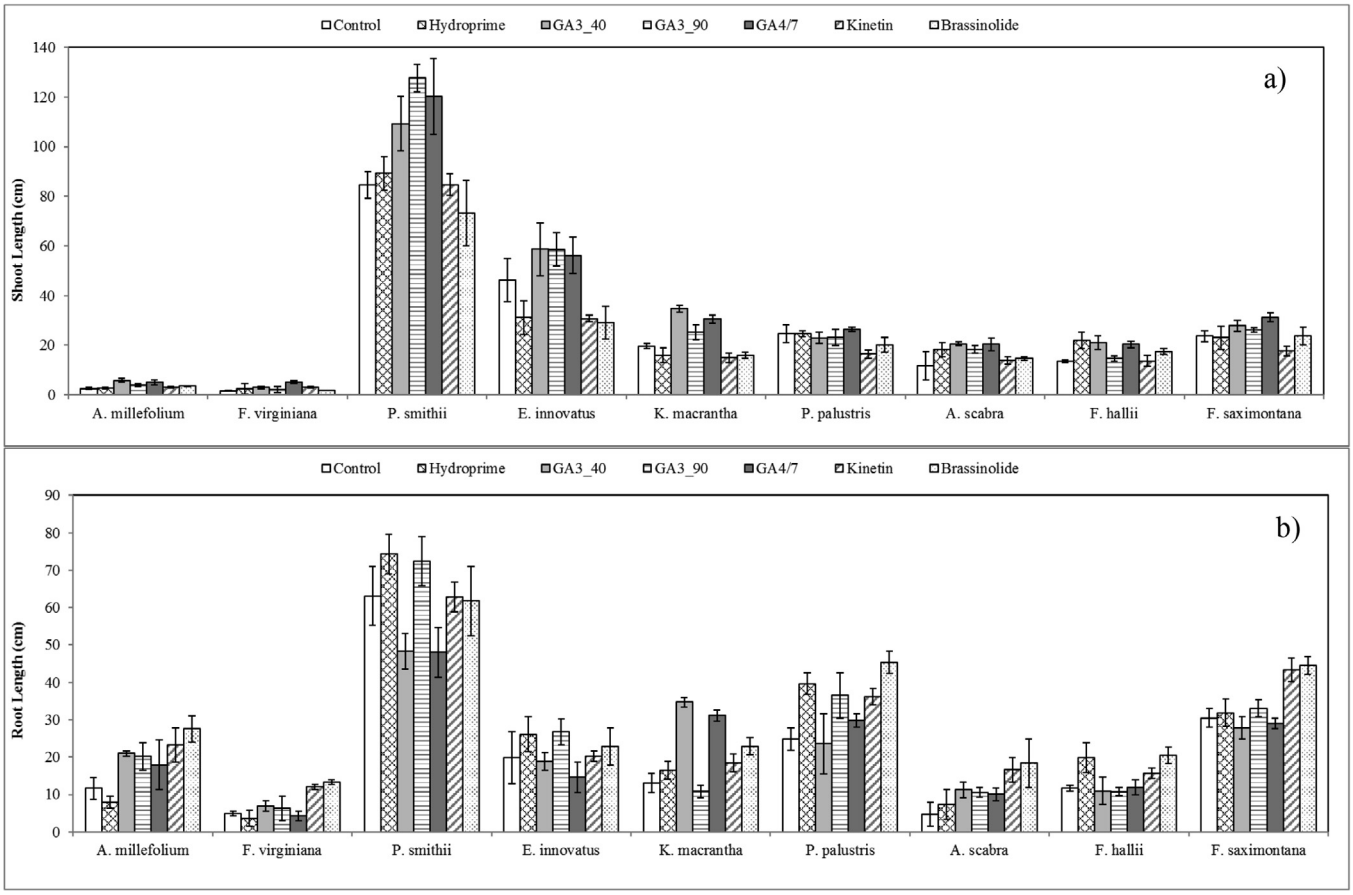

Fig. 1. Effect of plant growth regulator (PGR) treatments on forb and grass species a) shoot length, b) root length on day 14. Values are an average ± standard deviation (n = 100).

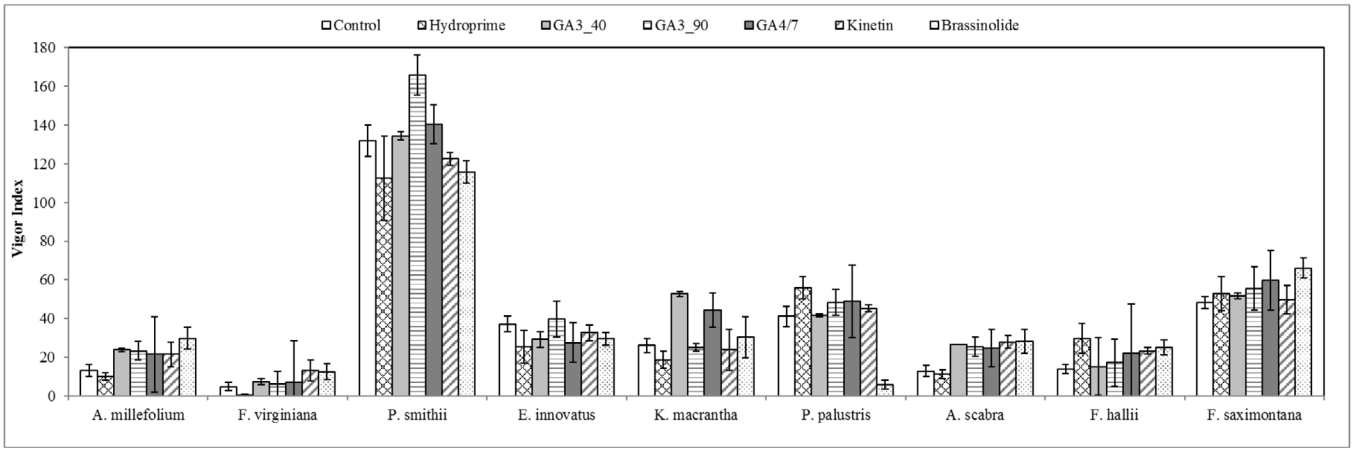

Fig. 2. Effect of plant growth regulator (PGR) treatments on forb and grass species vigor index on day 14. Values are an average ± standard deviation (n = 100).

on the native grasses and forbs tested within the experiment, indicating that there is the potential for their use as a seed treatment in re-vegetation and reclamation. Their potential benefits such as breaking of seed dormancy, improvement of early seedling emergence, and enhancement of shoot length is anticipated to provide native plants a competitive advantage over aggressive non-native species, which may ultimately reduce the time required to reach a self-sustaining plant community. Enhancing root development may assist in erosion control efforts, enhancing soil stability. Revegetation using native plants is considered successful when there is healthy and vigorous aboveground growth and plants are considered to have healthy root systems (Smreciu et al., 2003). Native grasses have slower establishment rates in comparison to weeds and agronomic species; therefore, an increase in seedling vigor is expected to be important for their survival especially under non-ideal environmental conditions (Baker, 1958). An increase in thatch and litter accumulation on the surface of the soil, through increased annual aboveground biomass production can also contribute to soil organic matter input which will enhance soil nutrient cycling, build soil structure and improve soil water holding capacity. Overall, these benefits may assist in the ultimate goal of achieving resilient functioning ecological systems following land disturbance.

Currently, there are no commercial PGR products that have been

developed for Alberta-specific native forbs and grasses. This presents an opportunity to investigate the applicability of commercially available PGR products for reclamation using native plant species and the associated methodology for such application. Future work should investigate ideal application rates for those PGRs that show the greatest potential as a seed treatment, based on the species of interest in a revegetation scenario. Subsequently, a field trial would be ideal to test the efficacy of PGRs under natural environmental and climate conditions. Research should also investigate whether products can be developed for other species of interest such as trees or shrubs that have poor germination and/or high mortality during initial establishment. Methodology for seed treatment should also be investigated as part of technology deployment on an operational scale, as it is currently unknown whether rinsing and/or air drying after seed soaking would impact the efficacy of PGR products. Understanding the longer-term impacts of PGRs on plant development using seed treatment application would also be beneficial to elucidate whether the effects of PGRs will persist and yield long-term benefits to the plants.

Conflict of interest

The authors declare that there is no conflict of interest.

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