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Effect of pre-germinative treatments on *Nothofagus glauca* seed germination and seedling growth

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Abstract

Background: *Nothofagus glauca* (Phil.) Krasser (Nothofagaceae, "Hualo") is an endemic tree of the Mediterranean zone of Chile. The natural forests in this area have been severely fragmented as a result of human causes such as replacement by agricultural crops and fast-growing tree species. From 1975, these forests have declined from 900,000 ha to 145,000 ha, so it is categorised on the IUCN Red List as 'vulnerable'. In restoring this ecosystem, efforts should focus, in part, on the propagation of quality stock. However, information on propagation systems is still insufficient.

Methods: We aimed to analyse the effect of different pre-germinative treatments and sowing times on seed germination, and seedling growth and quality. The pre-germinative treatments were: (i) cold stratification; (ii) soaking in gibberellic acid (GA_3) and thiourea solution; and (iii) nursery cultivation, while the sowing times were July, August and September.

Results: A high germination capacity was achieved by: soaking the seeds in GA_3 solution irrespective of concentration; stratifying, irrespective of period; or soaking in 7.5 mg L^{-1} thiourea solution, values significantly varied from that of the control treatments. The sowing time was not relevant in terms of the percentage of germination or seedling development. Stratification at 5°C for 60 days produced the best quality indices for *N. glauca* seedlings but no significant differences were found in any of the morphological attributes tested as a result of the pre-germinative treatments.

Conclusions: The pre-germinative treatments significantly improved the germination and seedlings growth of *N. glauca*. Cold stratification at 5°C for 60 days is recommended as it produced suitable seedlings for field establishment. Gibberellic acid and thiourea did not produce important effects on seedling growth. Our results suggest the presence of endogenous physiological dormancy of the *N. glauca* seeds. The results of this study provide important information on propagation and nursery techniques of *N. glauca*, which can be used in restoration programmes.

Keywords: Stratification; gibberellic acid (GA_3); thiourea; sowing time; seeds; Hualo

Introduction

The Mediterranean zone of Chile contains the country's greatest diversity of native flora and fauna (Myers et al. 2000). This area also supports deciduous forests that are adapted to prolonged periods of dry summers. These forests play very important roles in the conservation of water and organic soil and in the biochemical cycle of carbon, and they provide a great variety of ecological niches and habitats for flora, fauna, and associated microbiota (Arroyo et al. 1996). However, the highest human population density in Chile is also concentrated in this zone and there is strong anthropogenic pressure on natural resources. This situation led to a reduction of forest cover in this region (Donoso and Lara 1996) and the species that the forests contain. Between 1975 and 2013, it is estimated that the extent of these forests

declined from 900,000 ha to 145,500 ha (Santelices et al. 2013a; Urzúa 1975). One example is *Nothofagus glauca* (Phil.) Krasser (Nothofagaceae) (Hualo), which is an endemic species of this zone and is classified on the IUCN Red List as vulnerable (Barstow et al. 2017), but is not currently listed as an endangered species by the Chilean Government (D.S. 151/2007 Ministerio Secretaría General de la Presidencia). Its natural range includes mid-elevations, both in the Andes Mountains and the Coastal Range, in the central zone of Chile (Santelices et al. 2013a). Unfortunately, the remaining forests are frequently damaged or destroyed by wildfires, which leads to temporary changes in the soil properties (Litton and Santelices 2003). Such changes favour the invasion of exotic species such as *Pinus radiata* D.Don, which is particularly problematic (Litton and Santelices 2002). *Nothofagus glauca* forests are likely to be affected by climate change (i.e. an increase in temperatures and prolonged periods of drought) so it is important to study and manage the remaining native vegetation in the area where the species is naturally distributed, which belongs to one of the 25 biodiversity hotspot conservation areas that have been declared (Myers et al. 2000). Restoration of this degraded ecosystem is a priority task and, for this, it is essential to understand how to successfully propagate and cultivate nursery plants.

Most species in the genus *Nothofagus* exhibit seed dormancy (Cabello 1987, 2004; Leon-Lobos and Ellis 2005; Wilcox and Ledgard 1983), although not all to the same extent (Wardle and Campbell 1976). Cold stratification and treatments with growth regulators are most commonly used to overcome this type of dormancy (Baskin and Baskin 2014; Hartmann and Kester 1999). In *Nothofagus* spp., stratification shortens the time to germination but this is highly variable between seedlots and species (Wardle 1984). It has been observed that *N. glauca* seeds submitted to cold and humidity treatments increased their germinative capacity (Donoso and Cabello 1978). Gibberellic acid is a plant hormone that is known to improve plant development and has been shown to break dormancy and increase germination in seeds of several genera (Bewley and Black 1982; Bewley and Black 1985; de Mello et al. 2009). There is also evidence that treatment with a solution of gibberellic acid can break the state of internal dormancy of the seeds in some *Nothofagus* species in Mediterranean environments (Cabello 2004; Cabello et al. 2016; Rocuant 1984; Santelices et al. 2011). Previous work has shown that either cold stratification or gibberellic acid treatments can improve seed germination of related species including *Nothofagus macrocarpa* (A.DC.) Vasq. & Rodr. (Cabello et al. 2016), *N. alessandrii* Esp. (Gordon and Rowe 1982; Rocuant 1984; Santelices et al. 2011), *N. antarctica* (G.Forst.) Oerst., *N. betuloides* (Mirb.) Oerst. (Gordon and Rowe 1982), and *N. obliqua* (Mirb.) Oerst. (Gordon and Rowe 1982; Rocuant 1984; Rowe and Gordon 1981; Shafiq 1981; Subiri 1997). Treatment with a solution of thiourea, a chemical promotor of germination (Baskin and Baskin 2014), has also been recommended to break the dormancy of seeds but its effectiveness in overcoming the latent dormancy in some *Nothofagus* (*N. alessandrii, N. dombeyi, N. obliqua, N. pumilio* (Rocuant 1984)) is unclear. Little information exists about the effects of these promotors on *N. glauca* seeds, and there has been no documented effect in the cultivation of plants. Thus, in order to make the germination process be more efficient (i.e. having a better quality of germinated seeds in the shortest period), it is essential to carry out some type of pre-germinative treatment, to provoke physiological changes in the seed in order to accelerate germination. This can be achieved by simulating natural conditions or with the application of chemical growth regulators (Bonner 2008).

Previous works on the cultivation of *N. glauca* indicated that plants must be protected from direct radiation (González et al. 2009; Santelices et al. 2013b). These authors suggest that plants with morphological attributes suitable for forestation can be produced using 50–65% of shade although plants of an acceptable size and of a moderate slenderness index can be obtained using shading levels ranging from 18–35%. In addition to shade, the application of a controlled-release fertiliser in doses of 7.5 to 10 g L^{-1} significantly improved the growth of *N. glauca* compared with 3–4 g L^{-1}, which is currently being used (Santelices et al. 2013b).

The objective of this work was to analyse the germination process of *N. glauca* seeds submitted to different pre-germinative treatments and to evaluate the subsequent growth of seedlings in nursery subjected to these treatments.

Methods

Seed collection

Nothofagus glauca seed were collected in March 1998, near the town of Empedrado (Talca Province, Maule Region, Chile). The trees selected for seed collection were at least 50-m apart to increase the likelihood of possible genetic variation. The seeds were collected from 15 phenotypically similar trees, placing a plastic mesh on the ground, and they were transported to the Universidad de Chile nursery in Santiago, where they were cleaned, characterized using a purity analysis, and the weight and moisture content were determined using standard methods (ISTA 2006). In addition, the viability was determined by a cut test, for which three samples from each set of 50 seeds were chosen randomly. The determined characteristics were: 98.2% purity, 7.9% moisture content, 2,087 number of seeds $kg^{-1,}$ and 66% viability. Seeds were stored in polyethylene bags at 4°C for five months, to be germinated the following spring.

Pre-germination treatments and laboratory cultivation

Seeds were selected based on their phenological and sanitary appearance. All seeds that were damaged or showed some external anomaly were discarded. The remaining seeds were placed in distilled water for 24 hours. Seeds that floated were considered non-viable so were separated and discarded. Only viable seeds were used in subsequent experiments.

All the treatments were carried out sequentially so that the germination process could be started on the same day, thus maintaining the same conditions for all of them. Seeds soaked in distilled water for 24 hours were used as the control treatment.

Cold stratification – seeds were mixed with wet sand (which had been treated previously at 150°C for 60 minutes) and placed in polyethylene bags in a refrigerator at a temperature of 5°C (± 1°C) for periods of 30, 45, or 60 days.

Gibberellic acid – seeds were soaked for 24 hours in aqueous gibberellic acid solutions at concentrations of 25, 50, 100, 200, 400, or 800 mg L^{-1}.

Thiourea – seeds were soaked for 24 hours in aqueous thiourea solutions at concentrations of 7.5, 15, or 30 g L^{-1}.

Seed germination was carried out in the dark in a chamber at 20°C located at the Laboratorio de Semillas of the Universidad de Chile for 34 days. A completely randomised experimental design of fixed effects was used, with three replicates for each treatment, and 25 seeds per experimental unit. The germination process was controlled on a daily basis and, after 34 days, evaluated for germination capacity (percentage of germinated seeds with respect to the total number of seeds sown and the maximum Czabator value (maximum ratio from cultivated germination percentage on day x, divided by x (Czabator 1962)). In addition, the germinative energy was determined (accumulated percentage of germination on the day when the maximum value occurs); and the energy period (number of days in which the maximum value occurs). Seeds were considered to have germinated when the emerging radicles were over 2-mm long.

Nursery cultivation using selected pre-germination treatments

Once the laboratory experiments were completed, the best treatments from each experiment were selected based on the level of germination (i.e., 60 days cold stratification, soaking in 800 mg L^{-1} gibberellic acid solution and soaking in 7.5 g L^{-1} thiourea solution, plus a control treatment). These treatments were applied to viable seeds, which were subsequently sown directly onto a seedbed in a nursery at the Justo Pastor León Experimental Center from the Universidad de Chile, located in the Maule region. The characteristics of the soil used in the experiment are the following: loamy sandy texture, acidic pH (5.5); 1.58 ppm of organic matter; 5.18 ppm of nitrogen content; 13.49 ppm of phosphorus; and 17.84 ppm of potassium. Sowing was carried out during the last week of September. In the nursery experiment, plants were cultivated with bare roots in 1.1-m wide seedbeds with 8 rows of plants and 33 plants per metre. During cultivation, the plants were protected with a plastic mesh of 50% shade. A randomised complete block design of fixed effects was used, with three repetitions for each treatment and 40 plants per experimental unit, obtained from the centre of the seedbed to avoid edge effects. After one vegetative growth season in the nursery (8 months), the morphological attributes of stem length (L), root-collar diameter (D), above-ground biomass (AB), below-ground biomass (RB) and total biomass (TB = AB + RB), were measured. With this information, the slenderness index (SI) and the shoot to root ratio (SRI) were calculated, according to the following formulae:

$$SI = L\ (cm) / D\ (mm)$$

$$SRI = AB\ (g) / RB\ (g)$$

Laboratory or nursery cultivation of control seeds during different seasons

Independent experiments were conducted to examine the effect of sowing season on germination. Viable seeds obtained by soaking in distilled water for 24 hours were sown in the nursery during the last week of July, August, and September. The same experimental designs were used here as in the laboratory and nursery cultivation experiments described above.

Data analysis

Analysis of variance (ANOVAs) and the comparisons of means were done using the GLM (Generalized Linear Model) procedure in SPSS for Windows V.18. Data were transformed when necessary to ensure the assumptions of normality and homogeneity of variance. The variables expressed as percentages were transformed into angular values prior to determining the effects of the ANOVAs. Data were transformed logarithmically in those cases where the variables showed dispersion. Average values that were considered significantly different were compared using the Tukey test at the 5% level.

Results

Pre-germination treatments and laboratory cultivation

Significant differences were observed in the germination capacity and the maximum value of the seeds as a result of the pre-germination treatments (Table 1). A high germination capacity was achieved by: soaking the seeds in gibberellic acid solution irrespective of concentration; stratifying seeds at 5°C irrespective of period; or soaking the seeds in 7.5 mg L^{-1} thiourea solution. Stratification of seeds at 5°C for 60 days also produced significant differences in the maximum value (seed speed germination).

The germination capacity and maximum value decreased as the concentration of thiourea increased, with the highest concentration of thiourea decreasing germination capacity to levels comparable with untreated seeds. Based on these results and in the limited amount of available seeds, three treatments (soaking the seeds in 800 mg L^{-1} gibberellic acid solution; stratifying seeds at 5°C for 60 days; or soaking the seeds in 7.5 mg L^{-1} thiourea solution) were used for the nursery experiment.

TABLE 1. Effect of pre-germinative treatments and sowing times on the germination of *Nothofagus glauca* (means ± S.E. values with the same letter are not significantly different, $p < 0.05$).

Treatment	Germinative capacity (%)	Maximum value (% per day)	Germinative energy (%)	Energy period (days)
Pre-germinative treatment in the laboratory (sowing time September):				
Control	18.7 ± 1.3 c	0.60 ± 0.03 de	17.3 ± 1.3	29 ± 2.9
Stratification 30 days	80.0 ± 2.9 a	7.62 ± 0.70 bc	37.3 ± 5.8	5 ± 1.0
Stratification 45 days	72.0 ± 14.4 ab	8.78 ± 3.11 b	33.3 ± 13.5	4 ± 0.3
Stratification 60 days	94.7 ± 3.5 a	27.33 ± 1.33 a	54.7 ± 2.6	2 ± 0.0
Gibberellic acid 25 mg L^{-1}	86.7 ± 1.3 a	3.28 ± 0.28 cde	65.3 ± 10.4	20 ± 1.4
Gibberellic acid 50 mg L^{-1}	85.3 ± 2.6 a	3.69 ± 0.20 bcde	60.0 ± 2.3	16 ± 0.6
Gibberellic acid 100 mg L^{-1}	92.0 ± 2.3 a	3.75 ± 0.38 bcde	69.0 ± 5.8	19 ± 1.3
Gibberellic acid 200 mg L^{-1}	96.0 ± 4.0 a	4.39 ± 0.38 bcde	78.7 ± 7.4	18 ± 2.9
Gibberellic acid 400 mg L^{-1}	84.0 ± 6.1 a	3.85 ± 0.75 bcde	56.0 ± 10.5	16 ± 3.7
Gibberellic acid 800 mg L^{-1}	97.3 ± 2.6 a	5.43 ± 0.19 bcd	81.3 ± 3.5	15 ± 0.5
Thiourea 7.5 g L^{-1}	92.0 ± 4.0 a	4.81 ± 0.52 bcde	58.7 ± 3.5	13 ± 2.1
Thiourea 15 g L^{-1}	50.7 ± 2.6 b	2.55 ± 0.21 cde	42.7 ± 9.6	16 ± 2.6
Thiourea 30 g L^{-1}	5.3 ± 3.5 c	0.18 ± 0.12 e	5.3 ± 3.5	31 ± 1.6
Sowing times for control seeds in the nursery:				
July	73.3 ±11.6 a	0.84 ± 0.13 a	72.5 ± 11.4	86 ± 1.2
August	59.2 ± 6.0 ab	0.70 ± 0.06 a	58.3 ± 5.4	84 ± 1.2
September	24.2 ± 5.0 b	0.47 ± 0.10 a	24.2 ± 5.0	52 ± 1.4

Pre-germination treatments and nursery cultivation
Stratification at 5°C for 60 days produced the best quality indices for *N. glauca* seedlings but no significant differences were found in any of the morphological attributes tested as a result of the pre-germinative treatments used (Table 2).

Cultivation of control seeds during different seasons
The highest germination of control seeds in the laboratory (73%) occurred in July (Table 1) but the energy level was much higher than that of the pre-germination treatments and even with untreated seeds. The percentage germination decreased significantly for later sowing times. The only significant effect of sowing time on nursery-grown plants was on root-collar diameter (Table 2).

Discussion

The results obtained in this study indicate that *N. glauca* exhibits internal dormancy. This is consistent with findings reported by others for the same species (Donoso and Cabello 1978; Santelices et al. 2013a; Santelices et al. 1996), and for other *Nothofagus* species from South America (Arana et al. 2015; Cabello 2004; Cabello et al. 2016; Donoso 2013; Donoso et al. 2013), and Australasia (Fountain and Outred 1991; Wardle and Campbell 1976; Wilcox and Ledgard 1983). The reported germination percentages differ but this may be explained by differences in season of collection, the mast seeding habit of the species, and the geographical origin of seeds. Santelices et al. (2017) pointed out that patterns of seed germination in *N. glauca* are strongly influenced by provenance variability and suggested a potential

TABLE 2. Effect of pre-germinative treatments and sowing times on the morphological attributes and quality indices of *Nothofagus glauca* seedlings (means ± S.E. values with the same letter are not significantly different, $p < 0.05$).

Treatment	L (cm)	D (mm)	AB (g)	RB (g)	TB (g)	Quality index	
						SI	SRI
Pre-germinative treatment (sowing time September):							
Control	9.9 ± 1.2 b	2.96 ± 0.31 b	0.18 ± 0.04 b	0.21 ± 0.01 a	0.40 ± 0.05 b	3.4 ± 0.3 b	0.9±0.2 b
Stratification 60 days	22.1 ± 2.4 a	4.72 ± 0.44 a	0.87 ± 0.14 a	0.64 ± 0.16 a	1.52 ± 0.30 a	4.7 ± 0.1 a	1.7±0.1 a
Gibberellic acid 800 mg L^{-1}	16.1 ± 2.5 ab	4.06 ± 0.25 ab	0.60 ± 0.13 ab	0.56 ± 0.13 a	1.16 ± 0.26 ab	3.9 ± 0.3 ab	1.0±0.1 b
Thiourea 7.5 g L^{-1}	17.2 ± 2.4 ab	3.78 ± 0.27 ab	0.57 ± 0.13 ab	0.47 ± 0.12 a	1.04 ± 0.25 ab	4.5 ± 0.3 ab	1.2±0.0 ab
Sowing times for control seeds:							
July	8.0 ± 0.2 a	2.11 ± 0.02 b	0.08 ± 0.00 a	0.16 ± 0.01 a	0.25 ± 0.02 a	3.8 ± 0.1 a	0.6 ± 0.05 a
August	7.9 ± 0.9 a	2.09 ± 0.08 b	0.08 ± 0.16 a	0.16 ± 0.02 a	0.25 ± 0.04 a	3.9 ± 0.6 a	0.5 ± 0.04 a
September	9.9 ± 1.2 a	2.96 ± 0.31 a	0.18 ± 0.04 a	0.21 ± 0.01 a	0.40 ± 0.05 a	3.4 ± 0.3 a	0.9 ± 0.2 a

L= Stem length, D= Root-collar diameter, AB= Above-ground biomass, RB= Below-ground biomass, TB= Total biomass, SI= Slenderness index, SRI= Shoot to root ratio.

capacity of the species to adapt to climate. Dormancy and germination are important constraining factors that should be considered in restoration and reforestation programmes (Pérez-Fernández and Gómez-Gutiérrez 2007). For those species with seeds that show dormancy, it is advisable to develop effective strategies to ensure a greater and more homogenous percentage of germination and uniform seedling development. Previous work on related species (*N. macrocarpa, N. alessandrii, N. obliqua*) showed that cold stratification at 4°C (± 1°C) for 30, 45 or 60 days produced the greatest increase in germination percentage (Cabello et al. 2016; Santelices et al. 2011; Subiri 1997) while cold stratification at 5°C (± 1°C) for periods between 30 and 60 days were equally effective for *N. glauca.* Although cold stratification produced the best results, it requires more time as well as facilities that maintain cold conditions, so treatment with gibberellic acid or low levels of thiourea may be more cost-effective alternatives. Cold stratification for 60 days was the most efficient treatment tested as almost 55% of germinative energy was attained in an energy period of two days. Some doses of growth regulators generated lower maximum values than cold stratification but had higher germinative energy average values although with a longer energy period. In this context, the degree of difficulty of the treatments should be evaluated (GA_3 at concentrations higher than 100 mg L^{-1} are effective for the promotion of germination). In no case should the seeds be treated with doses equal to or greater than 30 g L^{-1} of thiourea, since concentrations greater than 30 g L^{-1} could negatively affect seed germination and seedling growth (Hartmann and Kester 1999). Although thiourea facilitates germination, its application in high elevated doses is not always accompanied by the necessary processes for normal radicle activity or for a normal cell division process, as it has been observed to inhibit the synthesis of DNA (Rodríguez et al. 1983).

Evaluating the effect of sowing season in terms of germinative capacity and maximum value of control seeds suggests that July and September may be the best months for sowing *N. glauca* seeds, although this result needs further research. However, the average germinative capacity observed was lower and the energy period was higher compared with the use of the pre-germinative treatments of cold stratification or soaking in gibberellic acid or thiourea. It is known that growth regulators are involved in the mechanisms that control the induction and breaking of dormancy (Leadem 1987) and with the exogenous application of these germination promoters, the dormancy is overcome quicker. Because of the variable masting/fruiting nature of the species, and due to the low seed availability during the season when the experiment was performed, only control seeds were used to test the effect of sowing season; however, future research should include the effect of different pre-germination treatments at each one of the sowing seasons.

Cold stratification is one of the most used treatments to break the internal dormancy of seeds of forest tree species (Baskin and Baskin 2014; Bonner 2008). A higher germinative percentage is obtained by stratifying the seeds for 60 days and the effect of this treatment is also reflected in seedling growth. All the morphological attributes we evaluated, with the exception of root biomass, were significantly different from the plants whose seeds did not receive treatment. Seedling height after one growing season (22.1 cm) was not exceptional but the diameter growth was 4.7 mm, which generated a slenderness index within the range suggested for species (like *N. glauca*) that live in Mediterranean environments (Villar-Salvador 2003). By observing the biomass partition and the shoot to root ratio, it is evident that the plants from this treatment tend to invest more in above-ground biomass than in below-ground biomass. There is not enough information about what SRI ranges may be optimal for the different Mediterranean forest tree species and there is no universal SRI associated with an optimal development of the plantations, but each species will have a range of optimal values (Villar-Salvador 2003). Thomson (1985) suggests a well-balanced SRI system for good seedling field performance, however, this contention must be taken with care for *N. glauca*. Therefore, it can be considered that the produced seedlings have desirable functional characteristics to be established in the field, although this hypothesis needs further research.

Gibberellic acid is a growth regulator that has been widely used as a promoter of seed germination in species with physiological dormancy (Baskin and Baskin 2014; Bonner 2008). In *Nothofagus betuloides* and *N. alessandrii*, it has been successfully used to break dormancy (Martínez-Pastur et al. 1994; Santelices et al. 2011). The latter authors, similar to that reported in our study, suggested low doses and short immersion times. However, there is a risk that plants with unwanted characteristics can be produced (Cabello 2004; Rascio et al. 1998), and this was observed in the first developmental stages of cultivated seedlings (e.g. gigantism, expressed by very elongated and succulent internodes). Nevertheless, there were no significant differences from the other pre-germinative treatments tested here, so pre-treatment with gibberellic acid could have a beneficial effect on the development of nursery seedlings. A similar situation was observed with the thiourea pre-treatment. In both cases, the morphological attributes of the seedlings obtained indicated that these plants also have suitable functional characteristics for forestation or restoration even though they were smaller than plants grown from cold-stratified seeds. However, according to the slenderness index, they could be somewhat robust, therefore they could have a lower growth capacity and survival due to the eventual deterioration of their carbon balance (Villar-Salvador 2003).

Thiourea has been less widely used than gibberellic acid for breaking seed dormancy and has been reported to inhibit root production of seedlings in some species (Baskin and Baskin 2014). There was less root biomass production with respect to the aerial biomass but a similar tendency as for the other pre-germinative treatments was also observed. It is noteworthy that, with the control treatment, seedlings invested more in below-

than in above- ground biomass. However, this result should be interpreted with caution since the difference in above- and below- ground biomass (i.e., 0.3 mg) of this treatment was very small and has little physiological significance.

The effect of sowing season for control seeds in the nursery was only significant for diameter. Seedlings were short and somewhat robust, thus would not be suitable for forestation or restoration. A similar, but not significant, trend was observed with seedlings whose seeds did not receive pre-germinative treatment, that is, to invest more in root biomass than in shoot biomass (in some cases close to 50%).

Conclusions

The results obtained in this investigation show that the seeds of *N. glauca* have an endogenous physiological dormancy, which can be overcome by cold stratification treatments (during 30, 45, or 60 days), with immersions in gibberellic acid (from 25 to 800 mg L^{-1}) and in Thiourea (7.5 g L^{-1}). With sowing at the beginning of winter (July), dormancy is also broken, although the germination capacity is lower than that achieved with the other treatments.

By stratifying the seeds at 5°C for 60 days, seedlings with functional attributes in order to be established in the field can be produced. However, with gibberellic acid and thiourea, no differences in the growth of the plants are observed. The sowing time did not have, in general, a significant effect on the development of the seedlings (except for the diameter) and the size of these plants is lower than that achieved with the other treatments.

The results of this study provide important information on propagation and nursery techniques of *N. glauca*, which can be used in restoration programmes.

Ethics approval

Not applicable.

Consent for publication

Not applicable.

Availability of data

Please contact the primary author for further information.

Competing interests

The authors declare that they have no competing interests.

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Author's contributions

Angel Cabello designed the experiment, supervised the fieldwork, and wrote and provided critical revisions of the manuscript. Nicolás Espinoza installed and assessed the experiments, and provided critical revisions of the manuscript. Sergio Espinoza evaluated the experiments, conducted the statistical analysis of the data, and provided critical revisions of the manuscript. Antonio Cabrera evaluated the experiments and provided critical revisions of the manuscript. Rómulo Santelices supervised the entire research, evaluated the experiments and wrote the manuscript. All authors read and approved the final manuscript.

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List of abbreviations

L= Stem length
D= Root-collar diameter
AB= Above- ground biomass
RB= Below- ground biomass
TB= Total biomass
SI= Slenderness index
SRI= Shoot to root ratio.